Agilent

Agilent 1290 Infinity III 2D-LC System MassHunter

User Guide

Notices

Document Information

The information in this document also applies to 1260 Infinity II and 1290 Infinity II modules.

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Contents

In This Book

This manual covers the Agilent 1290 Infinity III 2D-LC Solution MassHunter Acquisition for TOF/QTOF and TQ, or for High-End mass spectrometers.

1 Introduction

This chapter describes the product of 2D-LC Solution.

Introduction to the 2D-LC System

Product Description

The 1290 Infinity III 2D-LC System is an innovative solution for solving most complex separations, analyzing complex samples, and simplifying complex workflows. From separation of a few co-eluting compounds to mixtures of highest complexity - Agilent 2D-LC Solutions allow choosing from 2D-LC modes (multiple) heart-cutting with high-resolution sampling and comprehensive 2D-LC.

A wide range of applications in many industries benefit from orthogonal separations of samples, that cannot be resolved in one dimension at all or good enough within a short time. Comprehensive 2D-LC offers unmatched peak capacity for complex samples or sample matrices. 2D-LC can be used for desalting samples for salt sensitive separations or for making buffer-based separations MS compatible. In many cases, 2D-LC can be applied for simplifying workflows by replacing multiple 1D separations by one 2D analysis or by replacing offline fractionation through 2D-LC for faster, more reliable, and fully automated workflows.

The unique Agilent 2D-LC software with excellent ease of use makes 2D-LC available to everyone – from beginners, who want to create and review 2D-LC measurements in minutes up to experts using most advanced method development and data analysis capabilities.

Features

Agilent InfinityLab 2D-LC Solutions offers following key features:

- Agilent 2D-LC is based on 1290 Infinity III Systems with UHPLC performance, fast gradients, high sensitivity and excellent robustness.
- Dedicated 2D-LC valves use completely symmetric flow paths for reproducible retention times and peak areas.
- A wide range of modules can be used in both dimensions. Third-party detectors are supported via UIB II including the use of compatible detectors for data analysis.

- Powerful Agilent 2D-LC software is available for OpenLab CDS, MassHunter and ChemStation. Measurements can be set up easily with a few mouse clicks: Starting with a 1D measurement, choose spots where you want to increase resolution and draw your second dimension gradient.
- Agilent 2D-LC instrument control is fully automated and eliminates the need for tedious manual valve programming. Separation in the first and second dimension are completely independent by using Agilent multiple heart-cutting valves for highest storage capacity for up to 12 cuts at one time, fast and parallel analysis.
- High-resolution sampling is available for flexibly analyzing short cuts to broad peaks while retaining first dimension separation. By analyzing complete peaks, highly reproducible quantitative measurements can be achieved.
- Multiple heart-cutting and High-resolution sampling can be combined arbitrarily within one run.
- Shifted gradients, which can be edited graphically or numerically, maximize the available 2D separation space for highest peak capacity and fastest analysis.
- Dedicated flush gradients are available for fast analysis and minimum carryover.
- Cuts can be defined in time-based mode for highly reproducible measurements or peak-based mode for variable retention times or unknown samples. Even in peak-based mode, both first and second dimension detectors provide complete chromatograms.
- Dynamic peak parking combines time- and peak-based approaches for dealing with shifting first dimension retention times e.g. of biopharmaceuticals by using reference compounds.
- Multi-inject speeds up such analyses by sequentially injecting cuts from multiple sample loops.
- Smart peak parking optimizes runs for the highest possible number of cuts and shortest run time.
- A wide range of first and second dimension solvents and gradients can be combined with the optional Agilent Active Solvent Modulation Technology for multiple heart-cutting and high-resolution sampling measurements. By reducing first dimension solvent effects, second dimension separation is optimized and sensitivity is increased.
- The 2D-LC system can be prepared for a run by interactively and automatically flushing both dimensions' flow paths and all sample loops.
- The progress of a 2D run including parking of cuts in sample loops of deck valves and their analysis can be monitored in the dashboard.

- The 2D-LC system can be combined with analytical fraction collection.
- With an optional valve, switch easily between 1D and 2D separation.

GC Image Software

- GC Image LC x LC Edition Software for UV and single quadrupole or (Q-)TOF and QQQ detection is available from Agilent.

  It is used for visualizing 2D data and offers a sophisticated data analysis for comprehensive 2D-LC data including qualitative and quantitative results and statistical analysis.

LabAdvisor

- Diagnostic tests help with troubleshooting the 2D-LC system.

Terms Related to 2D-LC

Term	Definition
2D-LC	Two-dimensional liquid chromatography
1D	One-dimensional 1D-LC is the classical (one-dimensional) chromatography, which provides one-dimensional data. Usually, you would not even think about dimensions in the 1D world.
^{1}D	First dimension For example, a ^{1}D column is the column used in the first dimension, and a ^{1}D chromatogram is the chromatogram acquired for the first dimension.
2D	Two-dimensional 2D-LC is two dimensional chromatography, which provides two-dimensional data. Two-dimensional data is data that has a first and a second dimension. For example, 2D results can have chromatograms and peaks in each dimension. A 2D peak has a retention time in each dimension. 2D peaks are peaks in the two-dimensional contour plot.
^{2}D	Second dimension For example, a ^{2}D pump is a pump installed in the second dimension. The ^{2}D retention time is the retention time in the second dimension chromatogram, or the weighted averaged retention time for the second dimension in a two-dimensional peak. ^{2}D peaks are peaks in ^{2}D cut chromatograms.
2D compound	Two-dimensional compound, with a two-dimensional peak having a ^{1}D retention time and a ^{2}D retention time.
LCxLC	Comprehensive 2D-LC
LC-LC	Heart-cutting 2D-LC
MHC	Multiple Heart-cutting
HiRes	High-Resolution Sampling

2 Concepts of 2D-LC

This chapter describes the concepts of 2D-LC Solution.

Concepts of 2D-LC

In a 2D-LC-System, ^{1}D pump generates the ^{1}D gradient. An autosampler injects the sample and separates it by ^{1}D column. A 2D-LC Valve (Injector) connects the first dimension to the second dimension and stores sample peaks intermediately. These sample peaks are re-injected to the second dimension, separated by a ^{2}D column and measured by the ^{2}D detector.

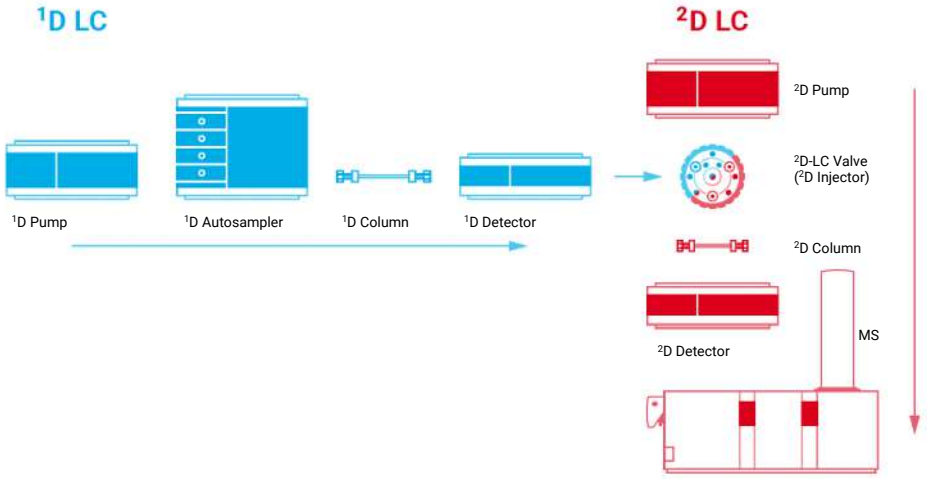


Figure 1: Concept of 2D-LC

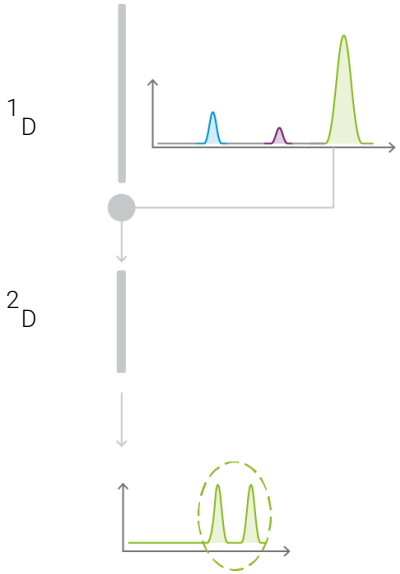


Figure 2: Conceptual illustration of heart-cutting 2D-LC principle

In 2D-LC the following concepts exist:

- Comprehensive 2D-LC (LC×LC)

  In LC×LC, the total eluent from the first dimension is injected on to the column in the second dimension.
- Heart-cutting 2D-LC (LC-LC)

  In LC-LC only parts of the eluent from the first dimension are injected on to the column in the second dimension.

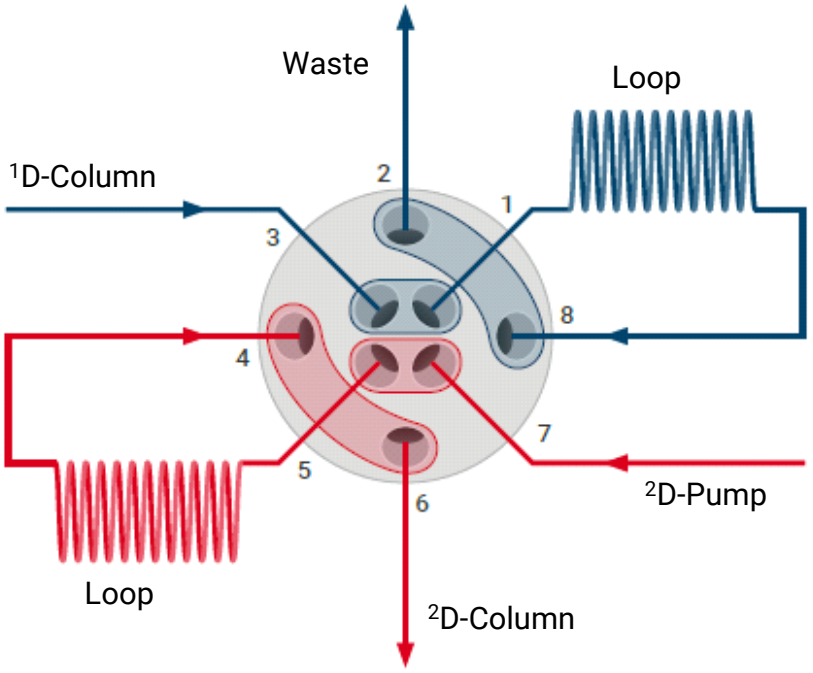


Figure 3: Standard 2D-LC valve (G4236A) with two loops (concurrent)

Heart-Cutting 2D-LC (LC-LC)

The following items are characteristic for LC-LC:

- Only parts of the effluent of the ^{1}D column - only the peaks of interest eluted from the ^{1}D column - are injected to the ^{2}D column
- A peak from the first dimension is sampled as a whole and a method with a lower flow rate and a gradient typically with a longer run time than the collection time is used to improve separation efficiency
- Typically longer columns with higher separation efficiency are used in ^{2}D column

NOTE Heart-Cutting 2D-LC (LC-LC) is the method of choice if the samples to analyze are known or to improve confidence of an experiment (pharma, method development and so on).

NOTE Multiple peaks eluted from the first dimension column can be sampled and analyzed in the second dimension but the run time of the second dimension must match the retention time between two first dimension peaks. *A started second dimension analysis will always be finished!* Thus, a second peak being eluted from the first dimension might be lost, if sampled while the second dimension analysis is still running.

Multiple Heart-cutting and High-Resolution Sampling 2D-LC

Typically, the gradient time in the second dimension is much longer for *heart-cutting* than with the *comprehensive* technique. The disadvantage of the standard heart-cutting techniques is that peaks cannot be sampled while a second dimension gradient is still running. In the examples shown here, the gradient from the second dimension is analyzing the first peak (purple), while the second and third peak (gray and yellow) elute from the first dimension column. The second dimension is ready when the 4th peak (green) elutes from the first dimension; this peak can be analyzed. As the second dimension is occupied again, the fifth peak (blue) cannot be analyzed.

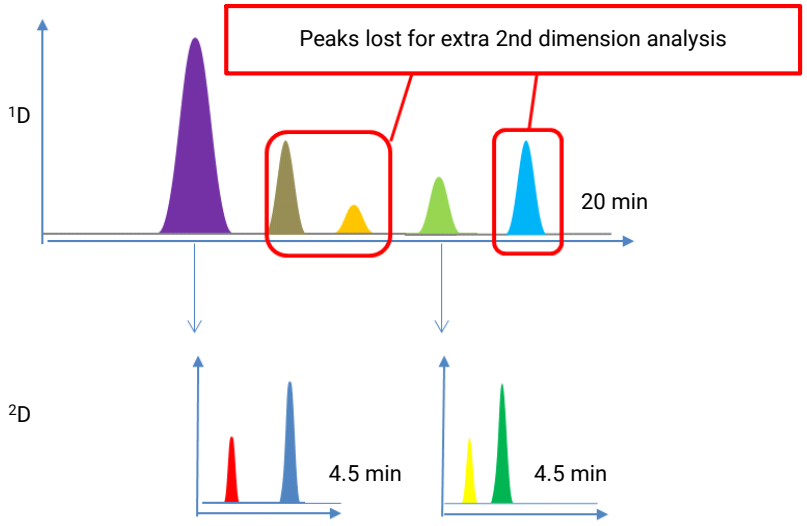


Figure 4: Example of lost peaks in Single Heart-Cutting

This problem is addressed using a setup called *multiple heart-cutting 2D-LC*. Here, the sampling loops on the 2D-LC valve are exchanged with 6-position/14-port selection valves, which are equipped with six loops each. In this configuration, a peak can be cut out and stored, then analyzed as soon as the second dimension is free.

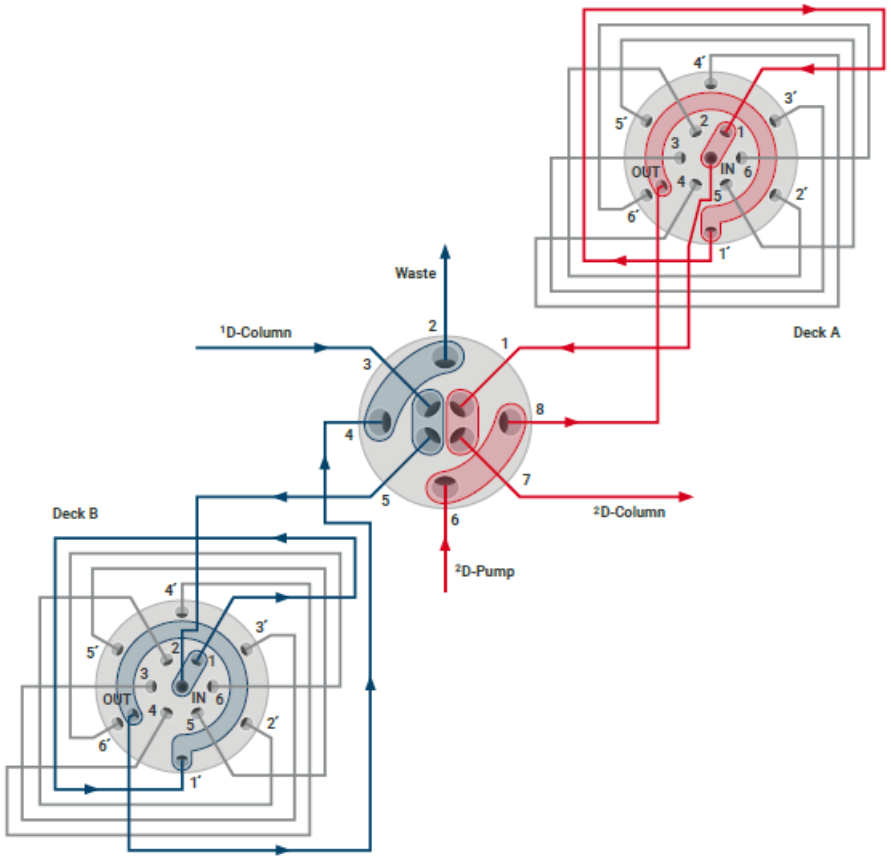


Figure 5: Standard 2D-LC valve (G4236A) with MHC 1300 bar (counter current)

Peaks that are cut out and stored during a run are analyzed consecutively in the second dimension, even when the first dimension is still running. To avoid carry-over the peaks are analyzed in reverse order of storage in a single Multiple Heart-Cutting Valve.

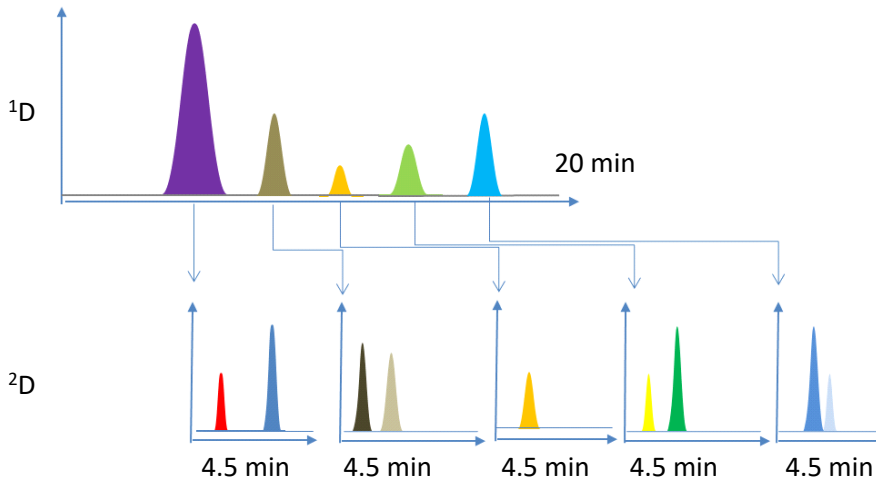


Figure 6: Example of a Multiple Heart-Cutting experiment with several cuts

Principles of Heart-cutting 2D-LC

Multiple Heart-Cutting - Principles

Multiple Heart-Cutting 2D-LC is a complex workflow, working on a special algorithm for filling the sample loops and analyzing the stored cuts, based on different criteria. The Multiple Heart-Cutting algorithm follows these principles:

- ^{2}D analysis is done as soon as possible. As long as the second dimension is free, any next cut from the first dimension will be always directly transferred to the second dimension and analysed. This means:
  - The first ^{1}D cut will be always directly analysed in the second dimension.
  - If the second dimension is free, when the next ^{1}D cut is taken, it will also be directly analysed.
- If the second dimension is occupied, the next ^{1}D cut will be stored in the next sample loop.
- If all sample loops in the first dimension are occupied, the peak is lost.
- A peak parking deck will always be completely analysed, before switching to the other parking deck.
- Before analyzing a new parking deck, a flush gradient is run to avoid contamination.
- Stored cuts are analysed in backwards order to avoid contamination.

Peak-based mode in multiple heart-cutting

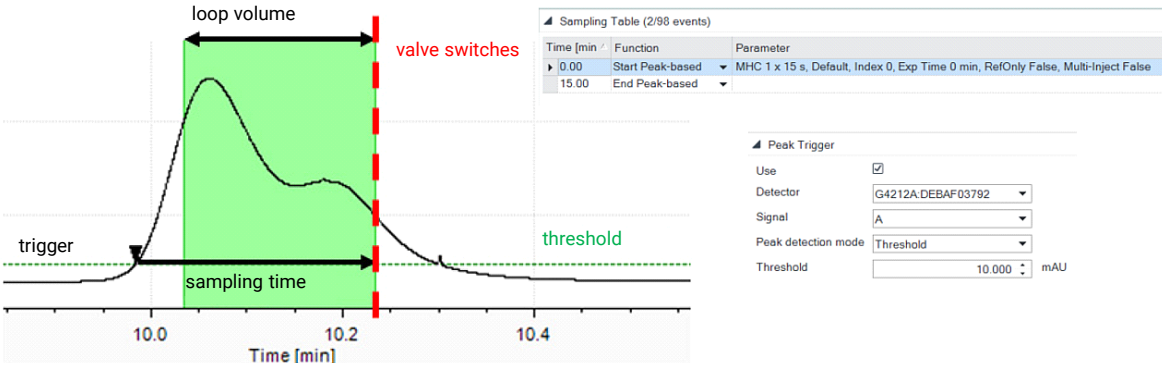


Figure 7: Peak-based mode

In peak based mode, three parameters determine how peaks are parked:

1. A trigger indicates, if a peak has been detected, e.g. because a reference signal (if available) exceeds the threshold or the slope as defined in advanced settings.
2. The cut is parked by switching the valve. This happens either if the peak end is detected (signal falls below threshold or slope) or if the settable cut size has been exceeded, whatever comes first. The purpose of the cut size is delaying the parking such that a defined part of the peak, typically its center, is parked.
3. The width t of the green area, which is used for parking a peak is fix and calculated from the loop volume V and flow rate F in the first dimension by t = V/F.

   NOTE: Please note that the peak parking may start even before the peak trigger if the sampling time is shorter than the time corresponding to the loop volume. In this case, the green area will start left to the trigger triangle.

Time-Based Mode in Multiple Heart-Cutting

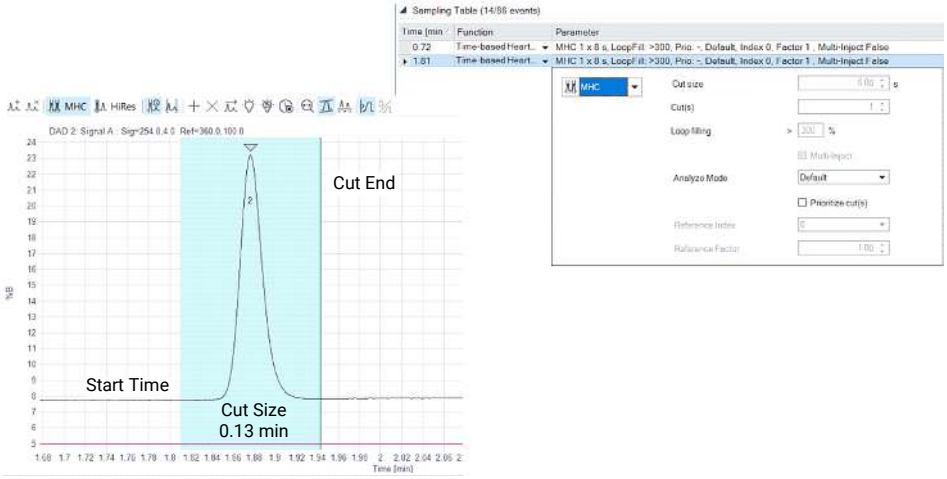


Figure 8: Time-based mode MHC

Time-based means that heart-cut times are defined in a timetable. This timetable can be constructed according to the first dimension retention time of peaks in a reference chromatogram. The time given in the sampling table corresponds to the beginning of the cut parking in the reference chromatogram. The cut size is fix and is given by t = V/F. The cut is parked by switching the valve at the time “cut end”. Ultimately, only the cut end has relevance for the method and instrument control. The cut end is displayed in preview as a reinforced line on the right side of the bar. If you want to move the cut you can do it graphically by moving the bar with help of the mouse or you can change the start time in the sampling table.

You can find more info in the chapter Method Parameters on page 152.

In MHC there is a limit of a few seconds on how close together you can place the individual cuts. This limit is primarily dependent on the required switching time of the 2D-LC valve. If you want to generate adjacent cuts you must use the High-Resolution mode, see High-Resolution Sampling - Peak Parking Principles on page 24. In multiple heart-cutting, loops should be overfilled (>100%). Please also note the cut size time is related to the flow rate. If the ^{1}D flow rate is changed, valve switch times are kept constant and the peak start time changes. Please note that the reference signal from the loaded reference chromatogram becomes invalid for a changed flow rate.

High-Resolution Sampling - Peak Parking Principles

In the HiRes sampling mode, the multiple heart-cutting (MHC) valve is switched *before and after* parking the peak. This has the following consequences:

- Each loop for consecutive snips stores the same sample volume.
- First and last loop cannot be used for parking.
- Solvent transfer from ^{1}D to ^{2}D can be reduced.
- Cut number 5 cannot be parked entirely in the sample loop. Otherwise cut 6 would got partially to the transfer capillary and would therefore be lost or spoil cut 5.

  Cut 5 stays partially in the transfer line and is immediately being analyzed in ^{2}D.
- For parking cut 6 in the sample loop, the cut first needs to be moved from the 2D-LC valve to the deck valve.

Peak parking example for HiRes sampling

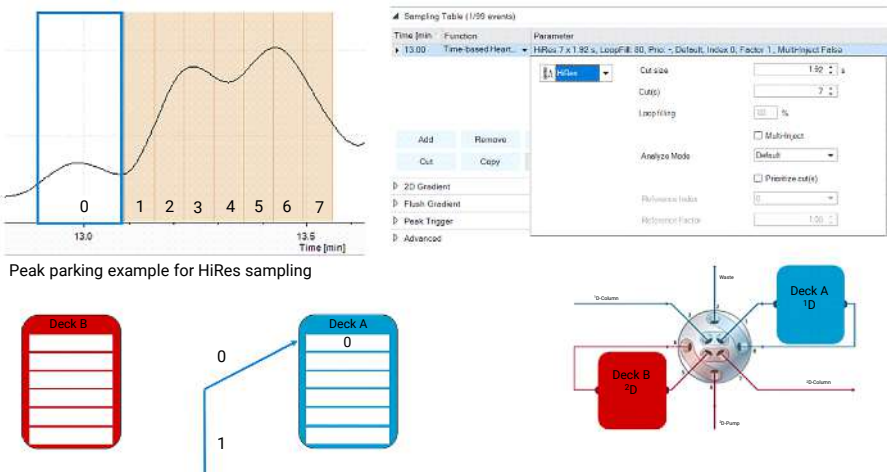


Figure 9: High-Resolution Parking principle

- In High-Resolution sampling, the first loop is a bypass position. When switching to the second loop for the first cut, unknown content may be parked in the first loop, which must be flushed at the end of the unparking procedure.
- MHC valve switches right before parking cut 1, 2, 3, 4, 5

- Cut number 5 cannot be parked entirely in the sample loop, otherwise cut 6 would go partially to the transfer capillary and would therefore be lost or spoil cut 5

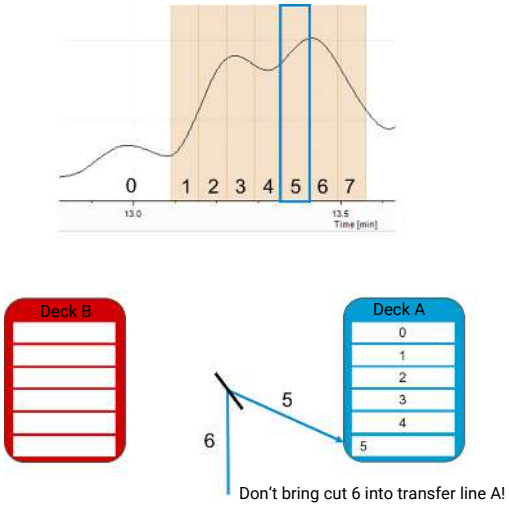


Figure 10: High-Resolution Parking principle

- Cut 5 stays partially in transfer line and is immediately analyzed in ^{2}D

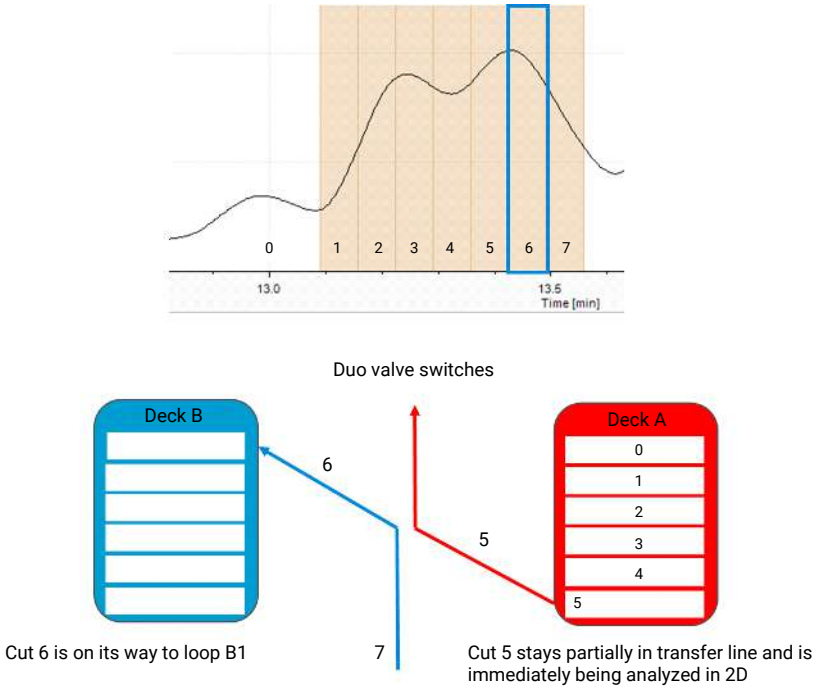


Figure 11: High-Resolution Parking principle with cut partially in transfer line

- For parking cut 6 into the sample loop, the cut first needs to be moved from the 2D-LC Valve to the deck valve.

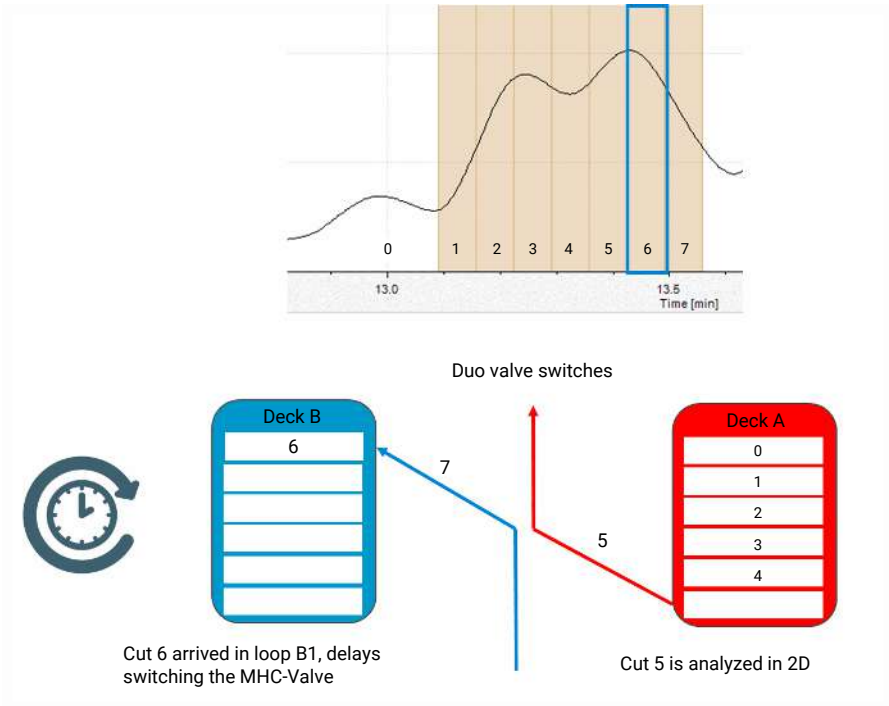


Figure 12: High-Resolution Parking principle with 2D-LC valve and deck valve

- Cut 7 will be parked in loop B2

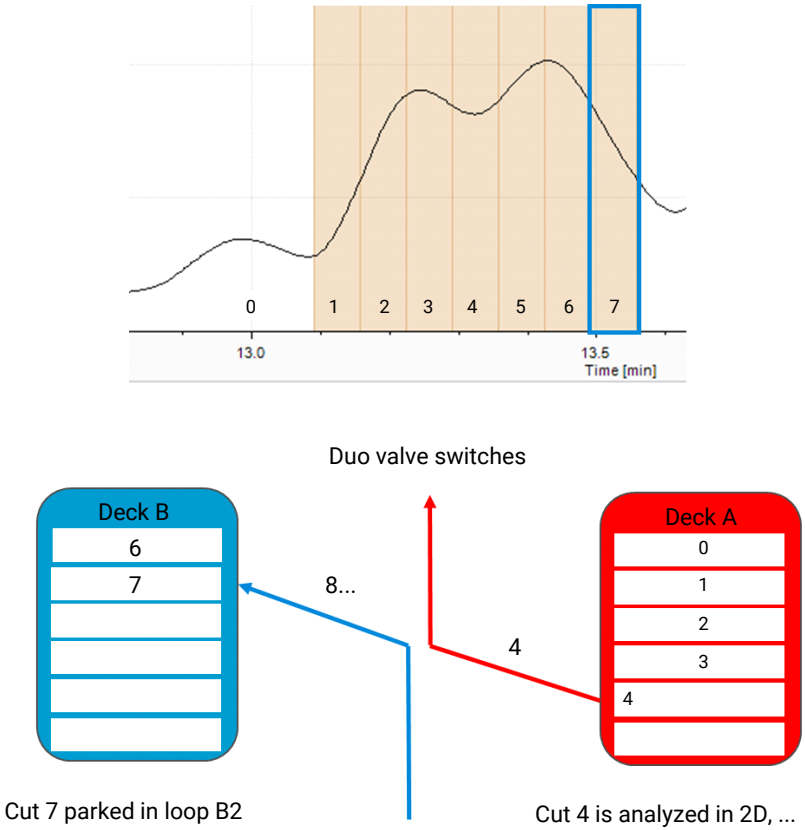


Figure 13: High-Resolution Parking principle with cut 7 parked in loop B2

- Last loop is required for flow-through while other deck runs analysis. During analysis, loops are filled with solvent of ^{2}D gradient base.

High-Resolution Sampling (time-based mode)

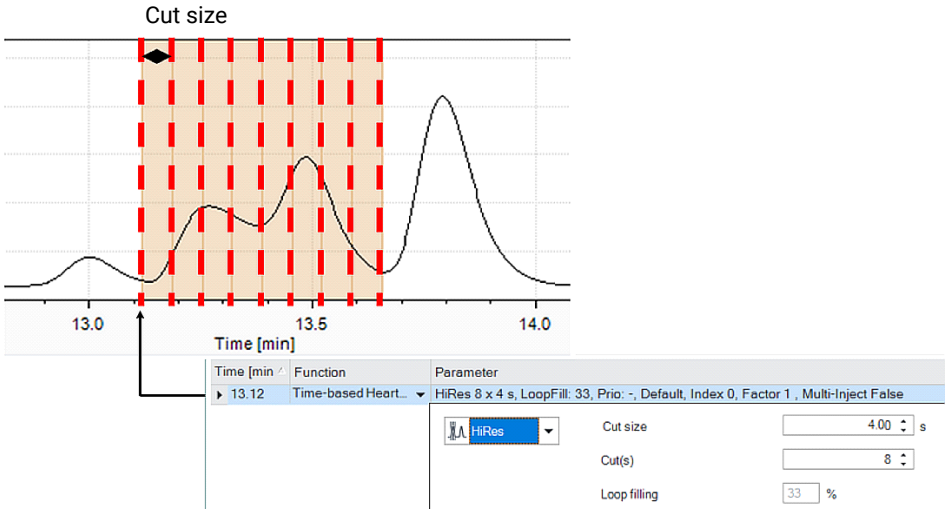


Figure 14: Comparison of High-Resolution Sampling in the chromatogram and the sampling table

For high-resolution sampling, a (start) time can be set, the cut size in seconds and the number of cuts for a peak or range. The sampling time should be less than the time which is needed for filling one sample loop corresponding to a loop filling below 80%. Because of the parabolic flow profile, a filling greater than 80% will cause samples going to waste.

The minimum cut size is given by the transfer volume between the 2D-LC valve and the deck valve. The last cut of a deck is stored in the transfer capillary such that switching to the second deck will bring that peak to the second dimension. If a volume smaller than that transfer volume would be chosen, two cuts would be in the same capillary resulting in a loss of resolution and reproducibility.

NOTE

Since the introduction of the driver-based 2D-LC solution, **HiRes** in peak-based mode is now also available.

Comprehensive 2D-LC (LCxLC)

In comprehensive 2D-LC (also known as LC×LC), the total eluent from the first dimension is injected on to the column in the second dimension using two equal-sized sampling loops that are alternated by a switching valve. While the first loop is being filled in the first dimension, the contents of the second loop is analyzed in the second dimension; the switching valve then switches the second loop into the first dimension for sampling and the first loop into the second dimension for analysis.

The gradient analysis in the second dimension is less than or equal to the cut size time in the first dimension:

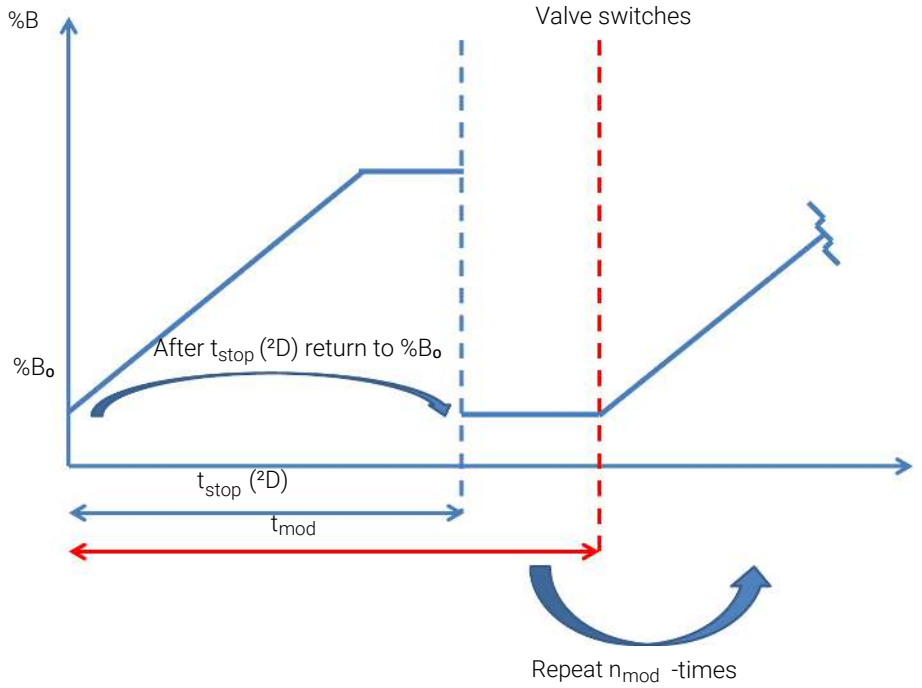


Figure 15: Characteristics of LCxLC

Standard LCxLC

In standard LCxLC the total eluent of the first dimension is injected onto the column in the second dimension using two sampling loops alternatingly by switching a modulation valve. This will be repeated from the start to the end of the first dimension separation.

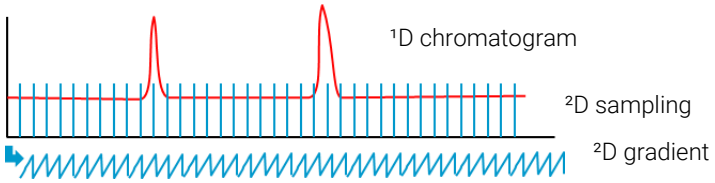


Figure 16: Principle of standard LCxLC

Triggering of 2D-LC

Concept of Peak Triggering

Peak triggered LC - LC

One or more peaks of the first dimension exceeding a given level are injected onto the ^{2}D column. Further peaks eluted from the ^{1}D column during the second dimension gradient time are ignored.

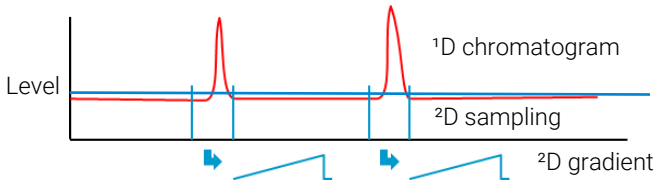
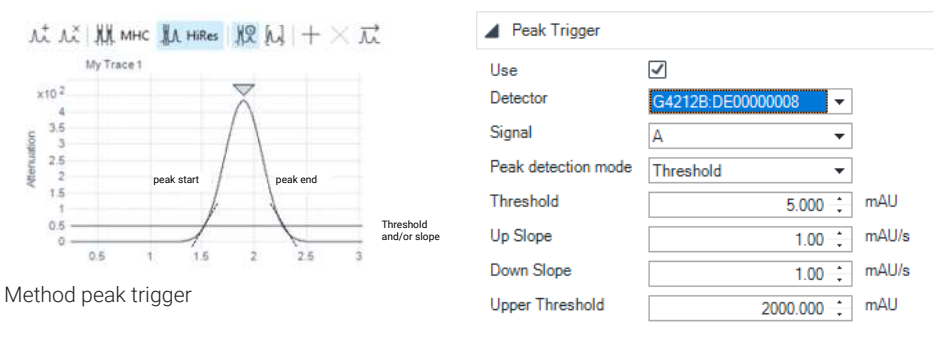


Figure 17: Principles of peak-triggered LC-LC

Relevant Parameters for Peak Triggering

Concept of Peak Triggering

Triggering is done in advanced settings similar to integrator settings by threshold and/or slope, see Use Peak Trigger on page 178.



Method peak trigger

The valve switches under the following conditions (whichever comes first):

- If the **Sampling time** has elapsed, or

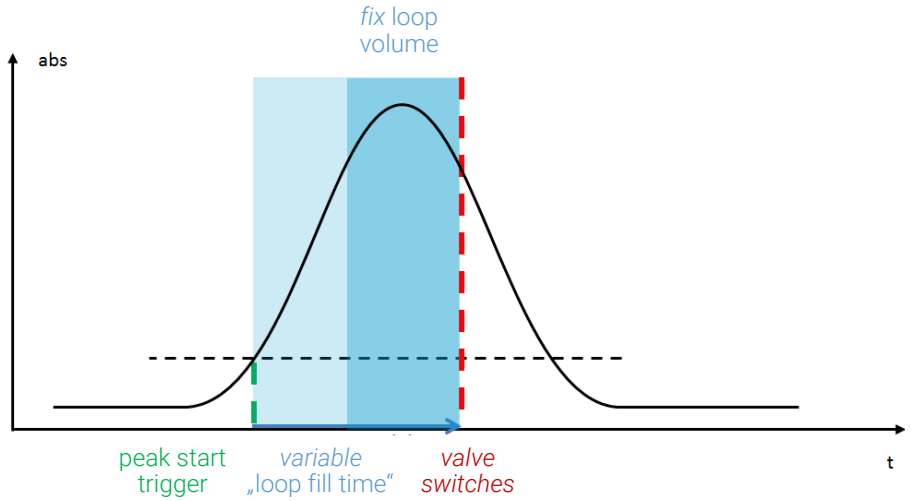


Figure 18: Peak triggering concept (elapsed sampling time)

- If the signal falls below threshold or slope.

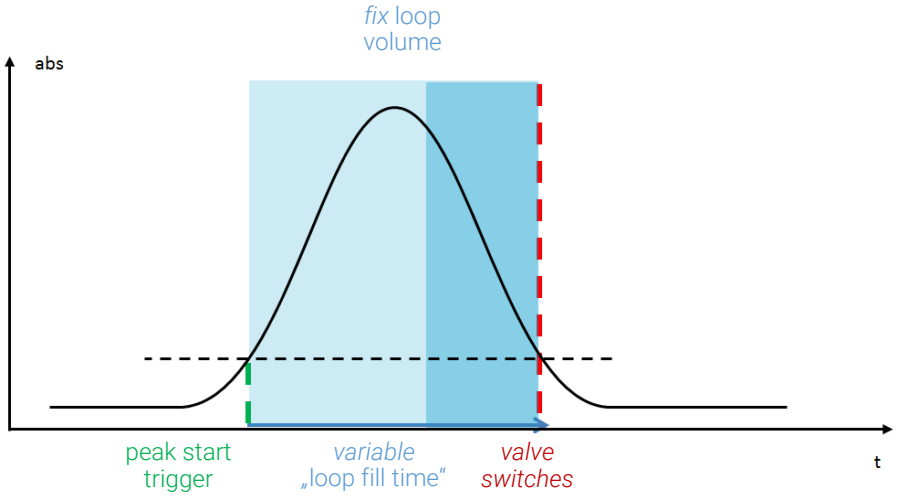


Figure 19: Peak triggering concept (signal falls below threshold or slope)

NOTE Compared to the 2D-LC Add-on in ChemStation, in the driver-based 2D-LC solution only the time triggering mode in LCxLC is available.

Concept of Time Triggering

Time-triggered LC-LC

One or more parts of the first dimension in given time frames are directly injected onto the ^{2}D column.

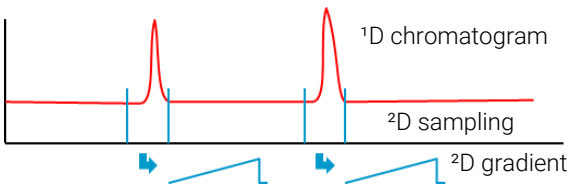


Figure 20: Principles of time-triggered LC-LC

Active Solvent Modulation (ASM)

Introduction to Active Solvent Modulation (ASM)

In conventional 2D-LC, ^{1}D solvent in the sample loop is injected to the second dimension column. If the ^{1}D solvent has high elution strength in respect to the ^{2}D column, it impairs separation in the second dimension. This results in unretained elution, broad and distorted peaks, and loss of separation (see Table 1 on page 37).

Active Solvent Modulation (ASM) dilutes the content of the sampling loop (sample and ^{1}D solvent) with weak ^{2}D solvent before it reaches the ^{2}D column and therefore improves the separation in the second dimension (see Table 1 on page 37).

Different ASM capillaries allow optimizing the dilution for different applications (see Understanding the ASM factor on page 41).

The ASM solution is primarily designed for 2D-LC modes multiple heart-cutting and high-resolution sampling. The 2D-LC Valve ASM is backward compatible to the standard 2D-LC valve G4236A. If ASM is not needed or for use in comprehensive 2D-LC, the ASM functionality can be disabled.

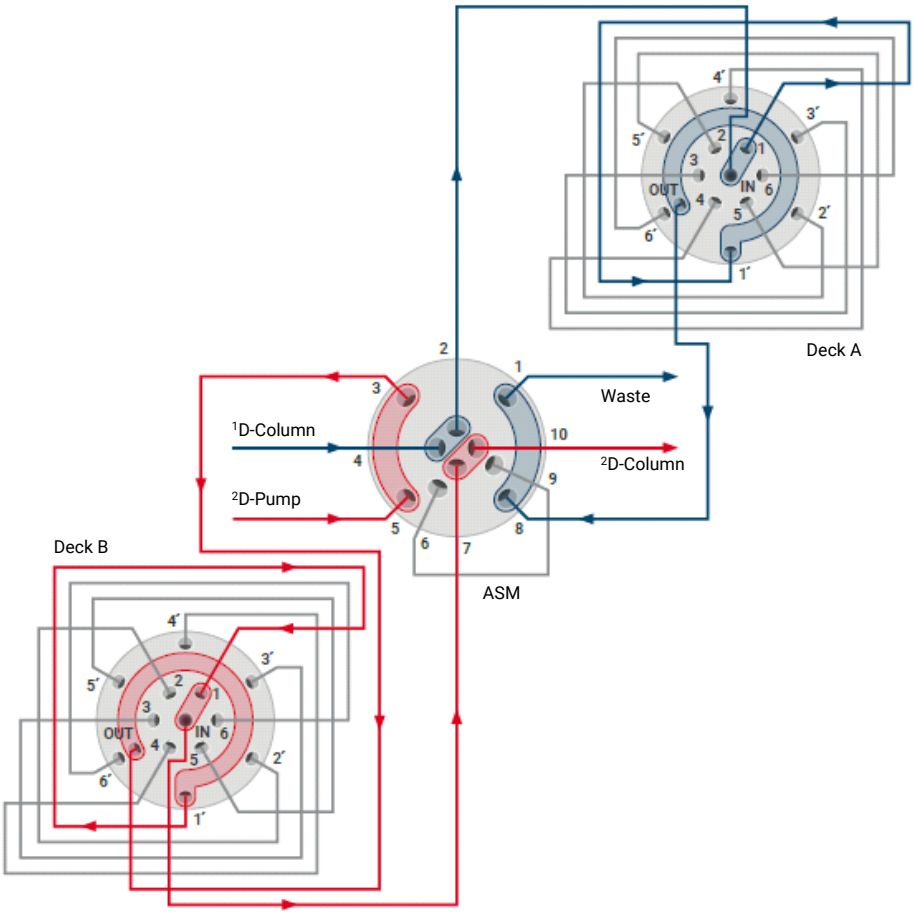
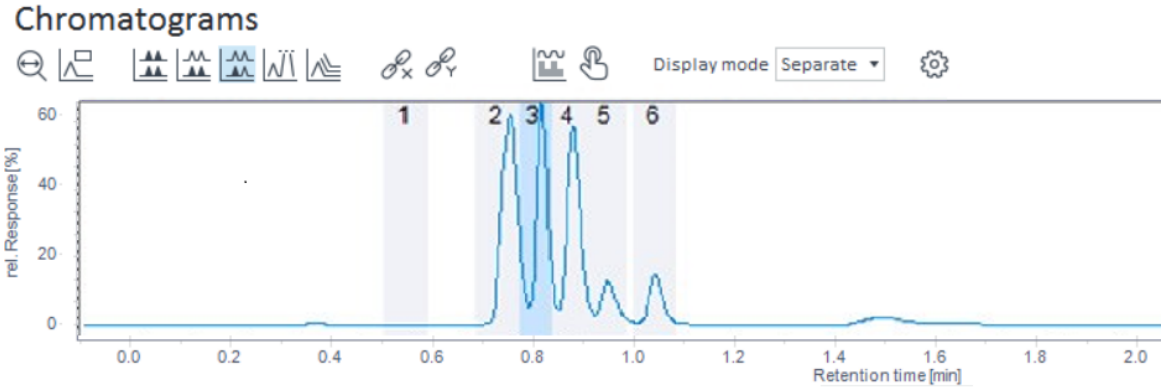


Figure 21: Schematic representation of the ASM 2D-LC Valve (G4243A) with MHC in countercurrent flow

Example: ASM with HILIC in [1]D and reversed phase in [2]D

In this example, a HILIC separation was run in the first dimension and a reversed phase separation in the second dimension. If sample cuts are transferred to the second dimension, 40 µL of high organic solvent are brought to a reversed phase column [1].

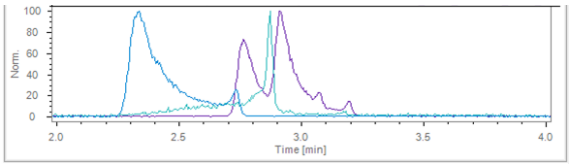
Table 1: Comparison of 2D resolution with conventional and ASM valves



Analysis of pesticides using a HILIC separation with high organic solvent composition in [1]D

2D resolution with conventional valve

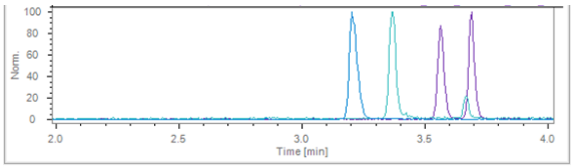
The high elution strength of [1]D solvent causes bad separation with broad and distorted peaks in the left [2]D chromatogram.



Conventional analysis of Cut#3 using a reversed phase separation in [2]D

2D resolution with ASM valve

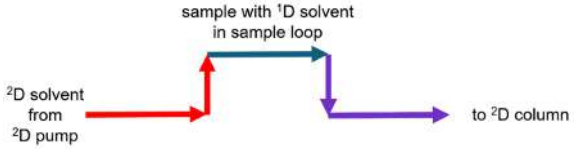
In the right 2D chromatogram a 2D-LC Valve ASM was used instead of a conventional 2D-LC valve. Peaks are resolved and the sensitivity is increased.



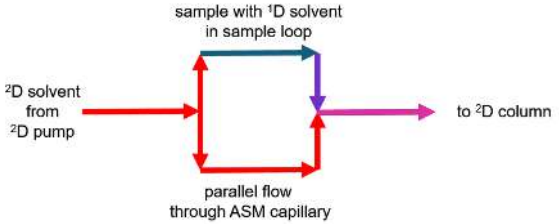
ASM analysis of Cut#3 using a reversed phase separation in [2]D

1 [1]D analysis of pesticides using: [1]D: Zorbax RX-SIL (150 x 2.1 mm ID, 5 µm), A = 10 mM NH_4Ac in H_2O; B = ACN, Gradient: 100 to 95% acetonitrile in 5 min, 500 µL/min. MHC with 40 µL loops. [2]D : Bonus RP (50 x 2.1 mm, 1.8 µm), H_2O/acetonitrile gradient (0.2% formic acid), weak solvent 3% acetonitrile, 400 µL/min, EICs from conventional 2D-LC (undiluted)

Operating Principle



Operating principle with sample loop in flow path (schematic view)



Operating principle with sample loop and ASM capillary in parallel flow path (schematic view)

¹D Solvent in the sample loop is partially diluted by ²D solvent from the ²D pump.*

Introducing a parallel flow through an ASM capillary strongly dilutes ¹D solvent with weaker ²D solvent. These solvent conditions focus the sample on the head of the ²D column and therefore enable a good separation.*

*red: ²D solvent from ²D pump, blue: sample with ¹D solvent in sample loop

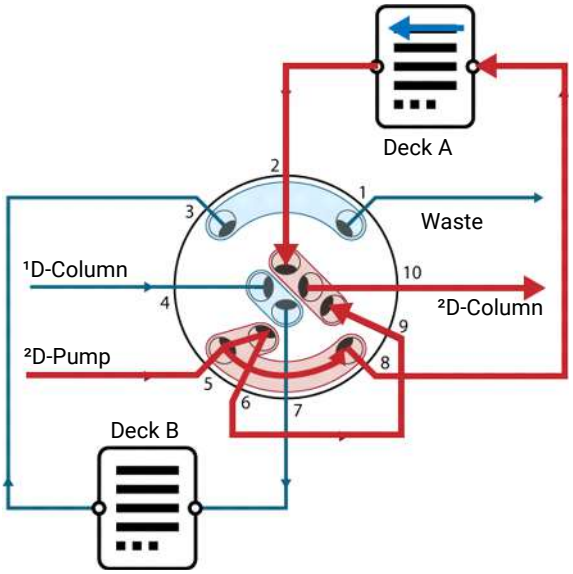


Figure 22: Operating principle with sample loop and ASM capillary in parallel flow path

This is how the same flow path looks inside the 2D-LC valve ASM. The flow coming from the ^{2}D pump splits up at valve port 10 . One part goes through the sample loop in deck A and carries parked sample cuts and ^{1}D solvent. The other part of ^{2}D solvent goes through the ASM capillary between valve ports 9 and 6 . Flows unite at port 5 and ^{1}D solvent is diluted before it arrives at the ^{2}D column head.

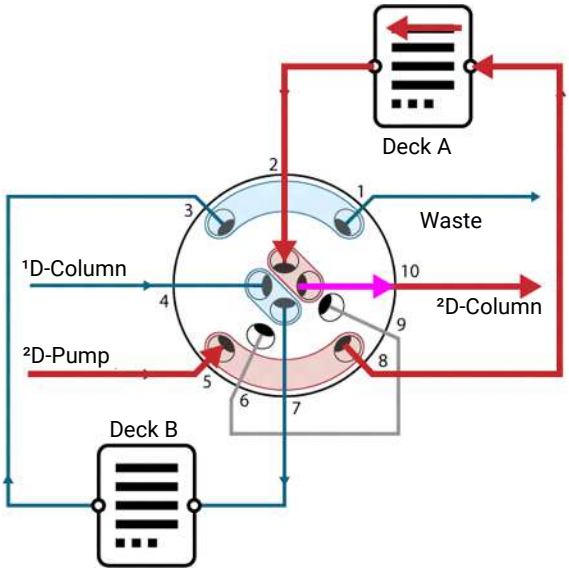


Figure 23: Operating principle with sample loop flow path

Once the ASM phase has finished, which is a settable method parameter, the analytical gradient starts. As opposed to a dilution with a permanent by-pass, the ASM capillary is no longer in the flow path, such that fast ^{2}D gradients are possible through the sample loop only.

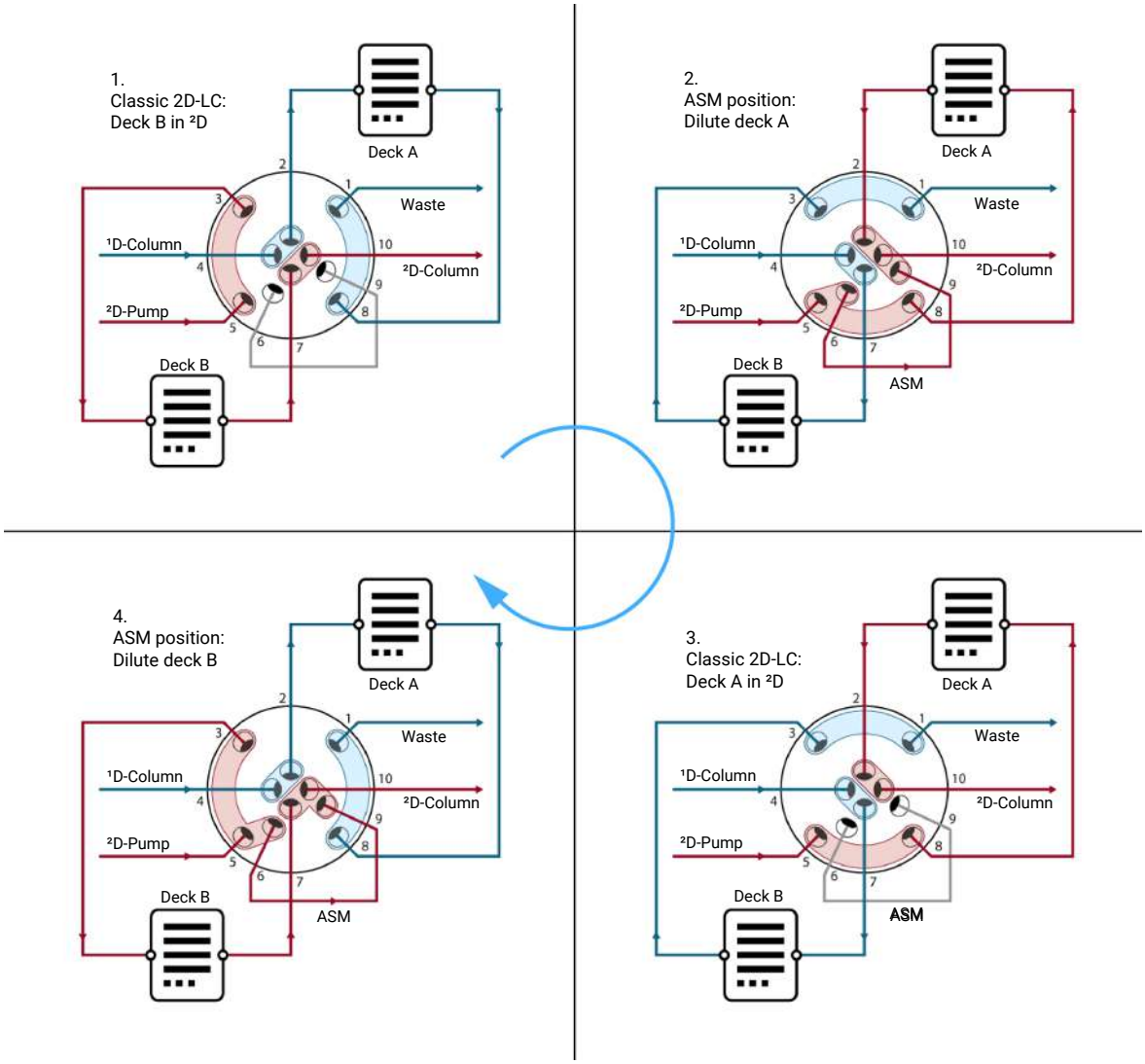


Figure 24: Switching cycle of the ASM valve (countercurrent mode)

Table 2: Switching cycle position names in the software (SW)

1 Classic 2D-LC: Deck B in ^{2}D

Position Names

Valve Position	Description
Position 1	Port 1 -> 8
Position 2	Port 1 -> 8 ASM
Position 3	Port 1 -> 3 -> 8 ASM
Position 4	Port 1 -> 3 ASM
Position 5	Port 1 -> 3

2 ASM Position: Dilute Deck A

Position Names

Valve Position	Description
Position 1	Port 1 -> 8
Position 2	Port 1 -> 8 ASM
Position 3	Port 1 -> 3 -> 8 ASM
Position 4	Port 1 -> 3 ASM
Position 5	Port 1 -> 3

4 ASM Position: Dilute Deck B

Position Names

Valve Position	Description
Position 1	Port 1 -> 8
Position 2	Port 1 -> 8 ASM
Position 3	Port 1 -> 3 -> 8 ASM
Position 4	Port 1 -> 3 ASM
Position 5	Port 1 -> 3

3 Classic 2D-LC: Deck A in ^{2}D

Position Names

Valve Position	Description
Position 1	Port 1 -> 8
Position 2	Port 1 -> 8 ASM
Position 3	Port 1 -> 3 -> 8 ASM
Position 4	Port 1 -> 3 ASM
Position 5	Port 1 -> 3

A full switching cycle of the ASM valve has 4 positions. Positions 1 and 3 are the same as for the standard 2D-LC valve G4236A. The ASM valve has two additional positions in step 2 and 4 . In both steps, the ASM capillary is in the second dimension and dilutes solvent in deck A and B, respectively.

NOTE Position 3 (Port 1 > 3 > 8 ASM) in the UI can be used to flush all lines in the ASM Valve.

Understanding the ASM factor

The principle of ASM is diluting ^{1}D sample loop solvent with ^{2}D solvent.

The ASM solution achieves this dilution by a parallel flow of solvents via sample loop and ASM capillary.

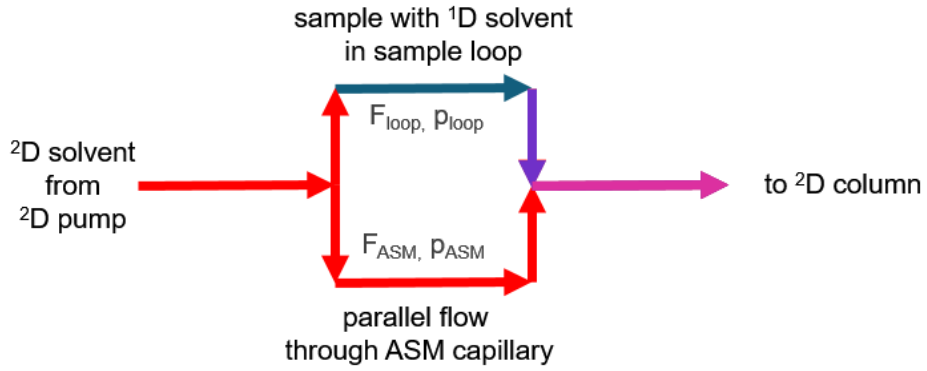


Figure 25: Principle of active solvent modulation (schematic view)

The flow rates *F* through these parallel capillaries depend on the different backpressures *p* of the capillaries in use. The backpressure of a capillary depends on the capillary length *l*, radius *r* to the power of 4 , and the viscosity *h* of the solvent.

$$p = \frac{8\eta l F}{\pi r^4}$$ Hagen-Poiseuille equation

The Hagen-Poiseuille equation describes the relation of these parameters.

Different ASM capillary lengths have an effect on the following parameters:

- Capillary back pressure
- Dilution factor
- Optimum dilution for different applications

Example for calculation of split ratio and ASM factor.

A longer capillary results in higher backpressure and therefore lower flow compared to a short capillary.

Example:

If the back pressure of the capillaries between ports 7 and 3 (2D-LC valve to sample loop and back) is twice as high as the back pressure of the ASM capillary between ports 9 and 6, twice as much solvent will run through the ASM capillary.

This will dilute 1D solvent in the sample loop by a factor of about 3, which is called the ASM factor.

NOTE

Usage of the ASM capillary kit results in the following situation:

- The capillaries in ASM branch and transfer branch have the same inner diameter.
- The two transfer capillaries are equally long.
- The difference between ID_{loop} = 0.35 mm and $ID_{capillaries}$ = 0.12 mm is large. Therefore the backpressure of the loops is negligible (this is, because the radius enters the Hagen-Poiseuille-Equation with the power of 4).
- Solvent composition and their viscosity in the parallel flowpaths are not predictable.

In the recommended configuration with the ASM capillary kit (see note above) one can simplify the formulae for the calculation of split ratio and ASM factor as follows:

$$Split\ ratio = \frac{l_{ASM}}{(2l_{tc1,2})}$$

l_{ASM} = Length of ASM capillary

$l_{tc1,2}$ = Length of transfer capillary 1 or 2

$$ASM\ factor = 1 + \left(\frac{1}{Split\ ratio}\right)$$

NOTE

The ASM factor calculated by the software should not be considered to be a fix number but as a guiding value which is subject to method development.

Comprehensive 2D-LC and Active Solvent Modulation

The ASM Valve can also be used for improving comprehensive 2D-LC measurements, but it is primarily optimized for multiple heart-cutting and high-resolution sampling measurements.

The ASM phase contributes to the modulation cycle. Keeping the modulation time constant, reduces the available time for the separation phase of the cycle. Otherwise, increasing the modulation time may require reducing the ^{1}D flow rate to fill the same sample loop volume. This would change ^{1}D chromatography.

The ASM solution requires backpressure from capillaries between the 2D-LC Valve to Multiple Heart-Cutting Valves. Therefore, comprehensive 2D-LC sample loops cannot be installed directly at the ASM valve. In addition, comprehensive 2D-LC sample loops have standard fittings, which do not fit to the M4 ports of the ASM valve.

Please note that ASM valves require twice as many switches as a standard 2D-LC Valve. Comprehensive 2D-LC switches the valve often and is therefore not recommended with ASM.

NOTE

Both, stator and rotor seal require regular maintenance. The wear of the ASM valve depends strongly on method parameters such as pressure and solvent (e.g. High buffer solution) therefore it is recommended to check the valve regulars with LabAdvisor.

3 Compatibility Matrix

This chapter provides information about installation and execution prerequisites regarding hardware, firmware, and the operating system.

MassHunter Workstation Data Acquisition

Following revision of MassHunter Workstation Data Acquisition is recommended:

- MassHunter Workstation Data Acquisition 11 for Q-TOF/TOF (or higher)
- MassHunter Workstation Data Acquisition 12 for TQ (or higher)

MassHunter Workstation Data Acquisition and High-End LC/MS instruments can be controlled with Agilent driver-based 2D-LC Solution. Please see the CDS_requirements in the CDS document folder which LC modules are supported.

This software has been tested successfully with 12 LC modules. Please note that complex systems can increase memory consumption in MassHunter, which may decrease system stability. This is very unlikely, but it is still advisable to consider the following:

- Restart MassHunter Workstation Software from time to time, e.g. once per week or more often for complex systems
- Perform data analysis, reporting, online help reading in an offline copy of the MassHunter instrument
- Save data before starting new tasks
- Avoid high levels of interactivity during runs by editing methods, changing signal plots settings, etc.

NOTE Update to MassHunter Workstation 11 or 12 requires a re-image of the PC.

NOTE Networked Workstation does not support file splitter.

Supported Operating Systems

Supported operating systems are the same as for the corresponding Agilent MassHunter CDS revision:

- Windows 10 Professional Semi-Annual Channel (64 bit) [1909 or newer]

- Windows 10 Enterprise LTSC editions (64 bit) [1809 or newer] Not shipped by Agilent
- Windows 11 Pro (or Pro for Workstations) General Availability Channel: 21H2 or newer
- Microsoft Office 365 Or Excel 2016 32 bit/Excel 2019 32 bit

For details, see the documentation of your Agilent MassHunter CDS edition like *Agilent MassHunter Workstation Requirements Guide (MHRequirements_EN.pdf, D0026036)* or *Agilent 1290 Infinity II 2D-LC System MassHunter User Guide (G2198-2D-LC-MassHunter-UseMa-en-D0028445.pdf, D0028445)*.

Available Languages

The embedded Agilent 2D-LC Software is available in English and has been tested with English versions of operating systems and CDSs.

NOTE Not all CDSs support all available languages. See the corresponding CDS documentation for further details.

General Software Requirements

Table 3: General Software Requirements

Component	Details
.NET framework	• NET 3.5.1 must be enabled on systems running on Windows 8.1 or Windows 10 or Windows 11, and • NET 4.7.2 or above (if needed, it will be installed automatically by the MassHunter Installer
Web browser	• Google Chrome 40 or higher • Edge
Anit-virus software [2]	• Microsoft Windows Defender

2 The listed anti-virus software has been tested to be compatible with the MassHunter software described in this document. While other thirdparty AV solutions may also be compatible, they have not been tested, and compatibility cannot be guaranteed.

PC Requirements

The following PC specifications for Agilent MassHunter Workstation are recommended.

Table 4: Tested and recommended hardware configuration for Workstations and Networked Workstations for TOF/Q-TOF

Item	For all LC/Q-TOF except 6546	For 6546 only
Description	Standard MassHunter-ready Computer	High Capacity MassHunter-ready Computer
Processor speed (CPU)	Intel Xeon W-2123, 4 core, 3.6 GHz	Intel Xeon W-2235, 6 core, 3.8 GHz
Physical memory (RAM)	32 GB	64 GB
Hard disk	1 TB M.2 NVMe SSD - Primary (C:\) Boot. 4 TB x 2 RAID1 (4 TB) - Data (D:\)	1 TB M.2 NVMe SSD - Primary (C:\) Boot. 6 TB x 4 RAID10 (12 TB) - Data (D:\)
Graphic Resolution	1920 x 1080	1920 x 1080
USB port [3]	1 USB port required for installation	1 USB port required for installation
LAN card - House LAN card - instrument [4]	Integrated Intel I217LM PCIe GbE Controller Integrated Intel I217LM PCIe GbE Controller	1 Integrated Intel I217LM PCIe GbE Controller 1 Intel Ethernet 210-T1 PCIe

Existing MassHunter Workstations with the Agilent bundled Z4 G4 PC are supported with MassHunter Workstation 12.0 running in Workstation configuration only.

Table 5: Minimum hardware configuration for Workstations

Item	For All TQ systems
Description	Hewlett-Packard Z4 G4 Minitower
Processor speed (CPU)	Intel Xeon W-2123 (3.6 GHz, 8.25 MB cache, 4 cores)
Physical memory (RAM)	16 GB (2 x 8 GB) DDR4 2666 DIMM ECC Registered Memory

3 If a USB port is available, the installation media can be copied over the network or downloaded from https://agilent.subscribenet.com.

4 A second LAN interface is required to isolate the instrument's data traffic from the local area network.

Item	For All TQ systems
Hard disk	2 x 500 GB 7200 RPM SATA 6G Hard Drive (RAID 1)
Graphic Resolution	1920 x 1080
USB port [3]	1 USB port required for installation
LAN card [4]	2 x Integrated Intel I219 and I210 PCIe GbE

For further Windows 10 and Windows 11 Configuration and Network Requirements, please refer to the *Agilent MassHunter Workstation Requirements Guide (MHRequirements_EN.pdf, D0026036)*.

Licensing

The Chromatography Data Systems used, by default require one or more licenses.

For more information about licenses, please refer to the documentation of the corresponding software. There it is described how a license is generated and installed in the control panel of the software. Usually the corresponding license authorization codes and/or license are included with each sales order. Additionally you need a USB hardware dongle to activate the 2D-LC solution.

For details, see Activate the 2D-LC System Driver With a License Dongle on page 110.

Supported Drivers

Table 6: Recommended and supported drivers

Firmware Set	Version of chromatographic data system	LC and CE Driver Version
A.07.02, B.07.35 C.07.30, D.07.35 (or higher)	MassHunter Workstation 11 (TOF/QTOF) (or higher) MassHunter Workstation 12 (TQ) (or higher) OpenLab CDS 2.7 (or higher)	3.5 (or higher)

Supported Firmware

Use the firmware, that is available in the Agilent 2D-LC Software USB flash drive in folder Firmware.

Agilent 2D-LC Software has been tested with following firmware revisions:

Table 7: Supported Firmware

Device	Firmware
Agilent 1100 Series, 1200 Series, and 1200 Infinity	A.07.02
Agilent 1200 Series, 1200 Infinity, and 1120 Compact LC	B.07.35
Agilent 1200 Infinity Hosted Modules	C.07.30
Agilent 1290 Modules	D.07.35
Agilent High-End LC/MS Instruments, e.g., G6546A	recent firmware [5]

NOTE

- Agilent releases LC firmware updates for so-called “firmware sets”.
- All Agilent LC instrument firmware sets have been designed and tested to be truly and strictly backwards compatible for the installed software base (CDS).
- The latest module firmware contained in each set is fully compatible and interoperable with all other module firmware of the same set.
- Agilent always recommends using the latest module firmware revision of a firmware set to avoid interoperatibility issues.
- Generally Agilent always recommends keeping the LC instrument firmware current.
- Do not mix firmware revisions between different sets. Agilent does not guarantee operation of mixed firmware revisions from older or newer sets.

NOTE

Firmware can be found and is available under the following link: https://www.agilent.com/en-us/firmwareDownload?whid=69761. Alternatively, see Replace the Module Firmware on page 324.

5 Recommended LCMS firmware: Always use the most recent firmware installation package that comes with the driver package.

4 Installation

This chapter describes the hardware and software installation of the 2D-LC Solution. The 2D-LC instrument can be used with the software described in this document. The installation instructions are valid for the modes standard heart-cutting, multiple heart-cutting, high-resolution sampling and comprehensive 2D-LC.

NOTE The 2D-LC solution only supports the instrument setup where the 2D-LC valve is hosted in the external valve drive, see General Information on page 57.

Hardware Installation of the 2D-LC System

Delivery checklist

Qty.	p/n	Description
1	G4243-90000	Agilent G4243A 2D-LC ASM Valve Guide Technical Note
1	5067-4266	2D-LC ASM Valve Head, 1300 bar
1	G4236-68000	2D-LC Easy Starter Kit (legacy) Internal part, not orderable
1	G4236-68100	2D-LC Easy Starter Kit for ESZ Service Internal part, not orderable
1	G1680-63721	Network LAN Switch
1	5500-1300	Capillary ST 0.12 mm x 85 mm M/M
1	5500-1301	Capillary ST 0.12 mm x 170 mm M/M
1	5500-1302	Capillary ST 0.12 mm x 340 mm M/M
1	5500-1303	Capillary ST 0.12 mm x 680 mm M/M
1	5500-1376	Capillary ST 0.12 mm x 170 mm M/M
1	5067-6171	Capillary Kit 2D-LC, Infinity Classic (optional) Internal part, not orderable
1	5067-6585	Capillary Kit 2D-LC, Infinity II/III Internal part, not orderable

The 5067-6585 (Capillary Kit 2D-LC, Infinity II/III) contains the following parts:

Qty.	p/n	Description
2	5043-0269	Adapter-profile for Agilent 1290 Valve Drive (G1170A)
1	5067-4608	Capillary ST 0.17 mm x 280 mm SX/S
2	5067-4651	Capillary ST 0.12 mm x 280 mm SL/SX
1	5067-4669	Capillary ST 0.12 mm x 600 mm S/SL
1	5067-4670	Capillary ST 0.17 mm ID 600 mm pre-swaged
1	5500-1217	Capillary, ST, 0.17 mm x 900 mm SI/SX
1	5500-1227	Capillary ST 0.17 mm x 150 mm SL-SL

Qty.	p/n	Description
1	5500-1240	Capillary ST 0.17 mm x 105 mm SL/SL
2	5500-1245	Capillary ST 0.17 mm x 400 mm SI/SI
2	5500-1251	Capillary ST 0.12 mm x 400 mm SL/SL

NOTE

Depending on the set up of you instrument, extra parts and capillaries might be required for instrument set up. Those parts are ordered separately or are shipped with other components, for example the 2D-LC or MHC valves. Their origin as well as their function is described in the instrument setup section below.

Options

NOTE

The 2D-LC System must contain an G7120A High-Speed Pump, G7132A Bio High-Speed Pump, or G4220A Binary Pump as ^{2}D pump.
This is necessary to achieve the following:

- Synchronize valve switches
- Run fast gradients on the ^{2}D column

Module identification: The module identifier (e.g. G7117A) can be found on the lower right side of the module front cover.

Table 8: Overview of recommended hardware configurations

Function	Functional Element	Part Number	Module	Comment
^{1} D	Pump	G7120A	1290 High-Speed Pump	
		G7132A	1290 Bio High-Speed Pump	
		G7112B	1260 Binary Pump	
		G7111B	1290 Quaternary Pump	
		G7104A	1290 Flexible Pump	
		G7104C	1260 Flexible Pump	
		G4220A/B	1290 Binary Pump	
		G4204A	1290 Quarternary Pump	
		G1312B	1260 Binary Pump	

Function	Functional Element	Part Number	Module	Comment
	Sampler	G7129B	1290 Vialsampler	
		G7167B/ G7137A	1290 Multisampler/1290 Bio Multisampler	
	Column Compartment	G7116B	1290 Multicolumn Thermostat	
		G1316C	1290 Thermostatted Column Compartment	
	Detector	G7117A/B/C	1260/1290 Diode Array Detector	Adjust the ^{1}D flow rate to the flow cell pressure specifications. See also the comment on the Pressure Release Kit. Recommended for multiple heart-cutting and high-resolution sampling as a peak trigger or for monitoring. Optional for comprehensive 2D-LC. ^{1}D flow cells require a minimum pressure stability of 60 bar (which excludes FLD and RID detectors).
		G7114A/B	1260/1290 Variable Wavelength Detector	
		G7115A	1260 Diode Array Detector WR	
		G7165A	1260 Multiple Wavelength Detector	
	NOTE: For ^{1}D/^{2}D Switching or time based measurements it might be necessary to use a mass spectrometer also in the first dimension. For further detail, see Alternative instrument setups for additional functionality on page 71.			
	NOTE: At the moment, non-CAN detectors, such as MSD and ELSD, can only be configured as second dimension detectors by the LC & CE drivers.			
Interface	Valve drive	G1170A	1290 Valve Drive	
	2D-LC Valve	G4236A	2D-LC valve kit, Standard	Contains the 2D-LC valve head
		G4243A	2D-LC valve kit, ASM	Contains the 2D-LC valve head with Active Solvent Modulation (ASM) functionality
	MHC Valves	G4236A#007 G4243A#007	Multiple Heart-Cutting Kit	Contains two MHC valve heads
		G4242A	2D-LC Multiple Heart-Cutting Upgrade Kit	Kit to upgrade MHC valves to an existing 2D-LC system

Function	Functional Element	Part Number	Module	Comment
	Pressure Release Kit (PRK)	G4236-60010	Pressure Release Kit	Mandatory if a [1]D detector is used. The kit prevents pressure pulses and protects detector flow cells!
[2]D	Pump	G7120A	1290 High-Speed Pump	1290 Infinity or Infinity II/ III Binary Pump required.
		G7132A	1290 Bio High-Speed Pump	
		G4220A	1290 Binary Pump	
	Column Compartment	G7116B	1290 Multicolumn Thermostat	Optional: A second column compartment is optional for large temperature differences between [1]D and [2]D. Any of these are supported as well as others or older modules.
		G1316C	1290 Thermostatted Column Compartment	
	Detector	G7117A/B/C	1260/1290 Diode Array Detector	
		G7114A/B	1260/1290 Variable Wavelength Detector	
		G7115A	1260 Diode Array Detector WR	
		G7165A	1260 Multiple Wavelength Detector	
		G1321B	1260 FLD	
		G4260A	1260 ELSD	
		G7102A	1290 ELSD	
			Agilent Single Quadrupole Detector LC/ MSD	
			High-End mass spectrometer like TOF/ QTOF or TQ	

NOTE: It is possible to connect third party detectors via UIB2 G1390A analog digital converter. But these third party modules have limited features in the CDS.
NOTE: Due to potential tailing, G7117A/B and G4212A/B Flow cells are not recommended for WCX and low salt SEC.
NOTE: To analyze photosensitive samples with UV-detectors (e.g. VWD, DAD WR, or LSS), prefer suitable flow cells and low light intensities. This is especially important for detectors in the first dimension.

Recommendations for Instrument Setup

General Information

InfinityLab 2D-LC Solutions come in several flavors, still allowing flexible HPLC combination of InfinityLab Series and 1200 Series Infinity modules. In combination with the Agilent Mass Spectrometer the HPLC part of the 2D-LC solution requires a two-stack configuration. For 2D-LC, a two-stack configuration is always preferred. On the left stack, the order of the modules from bottom to top is: pumps for both dimensions, then Vial- or Multisampler.

The sampler must be placed on top of the pumps. The right stack consists of one or two column compartments and one or two standard UV detectors.

Depending on the number of solvents used, both stacks offer the possibility to place a solvent cabinet on top.

Figure 26: Left: Recommended stack configuration for the 1290 Infinity III 2D-LC System. Right: Bench space requirements of the 1290 Infinity III 2D-LC System.

NOTE

The dual stack configuration for 2D-LC requires at least 97 x 62 cm (24.4 x 38.2 inches) free, vertical bench space. 2.5 cm (1.0 inches) of space on either side and approximately 8 cm (3.1 inches) in the rear is reserved for air circulation and electric connections.

Installation of the 2D-LC Valve and optional MHC decks

Attaching the external valve drives

For InfinityLab 2D-LC instruments that comprise at least one 1260 Infinity III or 1290 Infinity III pump, valve drives are attached to this pump with the 5067-5685 (Clamp Guide Kit) , while the valve drives are interconnected by the 5043-0269 (Adapter-profile) . The 2D-LC valve and if selected the MHC decks are mounted on external valve drives (G1170A).

#	Holders / connectors	Connection	P/N
3	1290 Infinity III Valve Drive (must be purchased separately)	Mounting of Valves	G1170A
1	Clamp Guide Kit (delivered with G1170A)	Top valve to pump	5067-5685
2	Adapter-profile (delivered with MHC Decks)	between G1170A drives	5043-0269

For a SHC configuration, the 2D-LC valve (G4236A) is attached to the upper pump of the stack. In case of a MHC configuration, the upper MHC deck is attached to the upper pump.

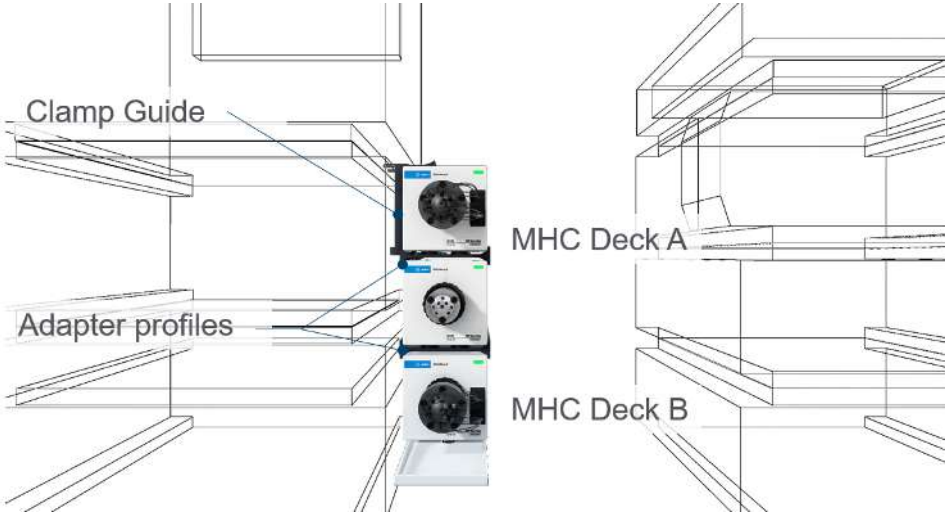


Figure 27: Schematic of the installation and attachments of the 2D-LC valve and optionally the MHC decks.

1 Mount the clamp guide on the right side of the pump: Markings in the form of round dips are on the body housing. Make a small hole with a peaked screw driver and tighten the clamp guide with the 3 self-cutting tapping screws.

2 Mount the valve heads on the G1170A external valve drives.

3 Clamp the first external valve drive with the MHC valve on top.

4 Attach the adapter-profile on each of the other external valve drives and mount them according to the positions shown in Figure 27 on page 58.

5 Mount the leak tray with sensor underneath the lowest external valve drive.

6 Install the Pressure release kit, see Installing the Pressure Release Kit on page 79.

Valve Configurations

Agilent InfinityLab 2D-LC Solutions offer two general valve configurations that decide which of the 2D-LC modes that can be used with the instrument. While the Single Heart-Cutting (SHC) configuration offers access to Single Heart-Cutting and Comprehensive 2D-LC, the Multiple Heart-Cutting (MHC) configurations additionally gives access to Multiple Heart-Cutting and High-Resolution Sampling 2D-LC. In addition, the Active Solvent Modulation valve (G4243A) is only available for the MHC configuration. An overview of all available 2D-LC modes can be found in Table 8 on page 54.

Stack setups of all other LC modules (reference) remain valid since those setups are independent of the valve configuration.

Table 9: Overview of 2D-LC modes dependent on valve configuration of the 2D-LC system

Valves	SHC Configuration	MHC Configuration
		
2D-LC Valve, Standard	✓	✓

Valves	SHC Configuration	MHC Configuration
2D-LC Valve, Active Solvent Modulation (ASM)	X	✓
Operation Modes	**SHC Configuration**	**MHC Configuration**
Comprehensive (LCxLC)	✓	✓
Single Heart-Cutting	✓	✓
Multiple Heart-Cutting	X	✓
High-Resolution Sampling	X	✓

Single Heart-Cutting Configuration

2D-LC instruments that are exclusively used for Single Heart-Cutting and Comprehensive 2D-LC experiments only require the standard 2D-LC valve (G4236A). The valve can be conveniently attached to any Infinity III pump that is installed. For a SHC configuration, transfer capillaries (6a/6b) are not necessary since MHC decks are not installed.

Figure 28: Schematics of a Single Heart-Cutting (SHC) Configuration with supported valves. For technical reasons, the ASM valve (G4243A) is not supported in Single Heart-Cutting setups.

Multiple Heart-Cutting Configuration

2D-LC instruments that are used for Multiple Heart-Cutting or High-Resolution Sampling 2D-LC require additional MHC decks. For MHC configurations, both the standard 2D-LC valve (G4236A) and the ASM valve head (G4243A) are supported. The valves can be conveniently attached to any Infinity III pump in the stack. Depending on the valve head that is used, different transfer capillaries (6a/6b) must be installed. For installation, please follow the guidance below.

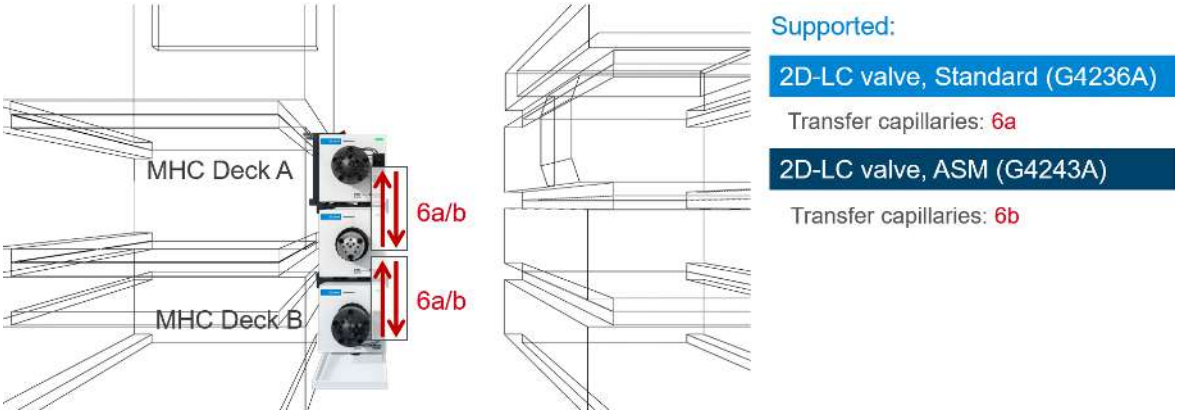


Figure 29: Schematics of a Multiple Heart-Cutting (MHC) Configuration with supported valves and transfer capillaries.

Recommended Stack Setups

InfinityLab 2D-LC Solutions allow three basic stack setups in three variations depending on the column compartment concept that is used. The pumps used for the first and second dimension distinguish the basic stack configurations. In the second dimension, a 1290 Infinity Binary Pump or 1290 Infinity II/III High-Speed Pump is mandatory. Agilent 1290 Infinity pumps are always based on the bottom. The capillary kit covers all recommended configurations. The following configurations optimize the system flow path, ensuring minimum delay and dispersion volumes:

Module identification: The module identifier (e.g. G7117A) can be found on the lower right side of the module front cover.

Table 10: Supported instrument configurations with a list of supported LC pumps. Numbers refer to the stack setup that is recommended.

#	[1]D pump	supported [2]D pumps
1	**1290 Infinity II/III / 1260 Infinity II/III Prime LC** 1260 Flexible Pump (G7104C) 1260 Bio Flexible Pump (G7131C) 1290 Flexible Pump (G7104A) 1290 Bio Flexible Pump (G7131A) 1290 High-Speed Pump (G7120A) 1290 Bio High-Speed Pump (G7132A)	**1290 Infinity / 1290 Infinity II/III** 1290 High-Speed Pump (G7120A) 1290 Bio High-Speed Pump (G7132A) 1290 Binary Pump (G4220A) See Figure 33 on page 68
2	**1290 Infinity** 1290 Quaternary Pump (G4204A) 1290 Binary Pump (G4220A)	**1290 Infinity II/III** 1290 High-Speed Pump (G7120A) See Figure 34 on page 69
3	**1260 Infinity Binary / 1260 Infinity II/III Binary** 1260 Binary Pump (G7112B) 1260 Binary Pump (G1312B)	**1290 Infinity II/III** 1290 High-Speed Pump (G7120A) See Figure 35 on page 70

NOTE

This guide only covers setups that contain at least one Infinity II/III pump module! Setups that contain exclusively 1200 Infinity Series modules must be installed with the corresponding capillary kit.

Connections mentioned in this setup are the following:

— Concurrent direction for the Standard 2D-LC Valve (G4236A) with Single Heart Cut Configuration, see Figure 30 on page 63.

— Countercurrent for the ASM 2D-LC Valve (G4243A) or Standard 2D-LC Valve (G4236A) with a Multiple Heart-Cutting Configuration, see Figure 32 on page 66.

⇨ In the instruction table, the connections to valve port are mentioned in brackets, for example ASM Valve (2) = ASM Valve, Port 2.

⇨ If you want to connect the 2D-LC Valve in another direction than in these recommended 2D-LC setups, please follow the schematics shown under 2D-LC Valve Topologies in the LC Driver Online help.

Connecting the 2D-LC Valve, Standard (G4236A)

The capillary connections of the 2D-LC valves depend on whether a con- or countercurrent configuration achieved. For the standard 2D-LC Valve, both concurrent and countercurrent operation is possible. Schematics in this chapter will reflect a concurrent direction.

If you want to connect the 2D-LC Valve in a different direction, follow the schematics shown under **2D-LC Valve Topologies** in the LC Driver Online help.

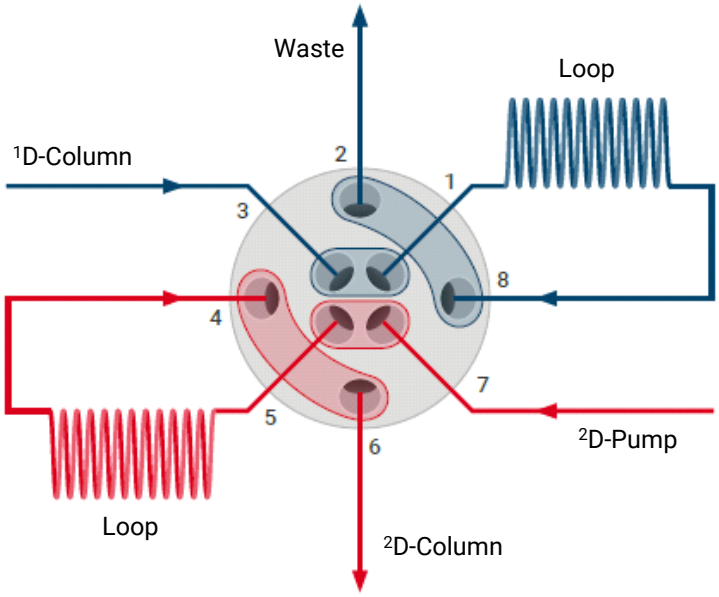


Figure 30: Schematic representation of the Standard 2D-LC Valve (G4236A) in concurrent flow.

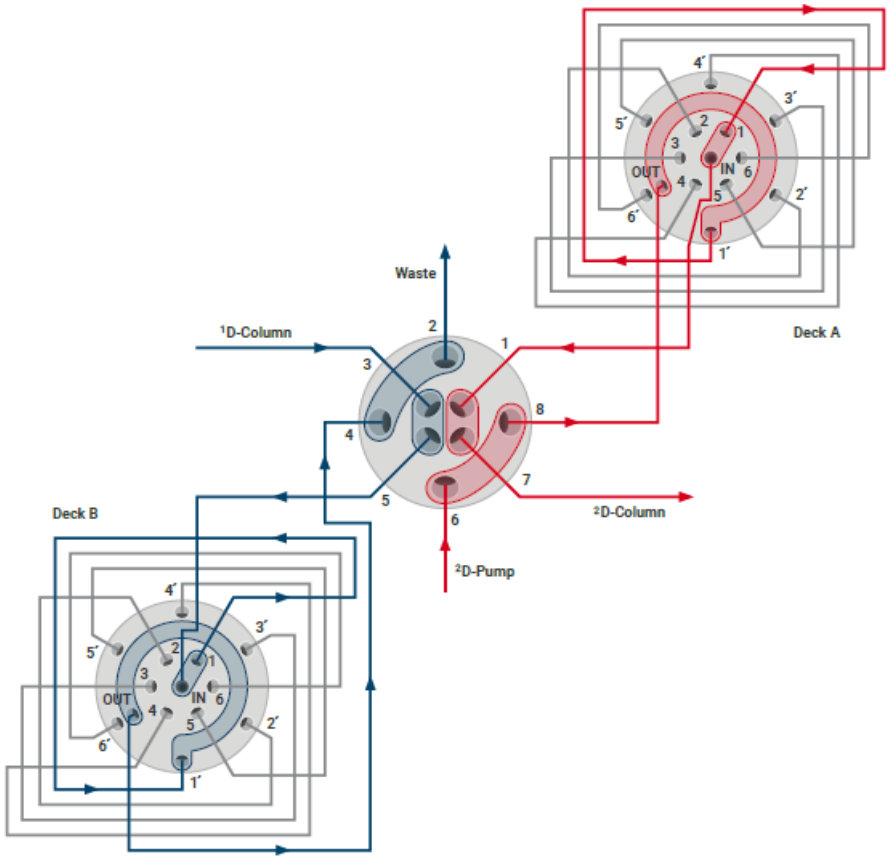


Figure 31: Standard 2D-LC valve (G4236A) with MHC 1300 bar (counter current)

Port	Number of Capillary	Connection	ID x L [mm]	P/N	Description
1	6a	transfer capillary to MHC Valve (OUT), deck A	0.12 x 170	5500-1270	Capillary ST 0.12x170 S/M
2	11	waste line	0.7 x self-cut	0890-1713	Tubing-flexible 0.8/1.61mm PTFE WT (delivered with UV detector)
3	5	from pressure release kit; from ¹D column, ¹D detector	0.17 x 105 0.12 x 500	5500-1240 5500-1157	Capillary ST 0.17x105 SL/SL Capillary ST 0.12x500 SL/S
4	6a	transfer capillary to MHC Valve (IN), deck B	0.12 x 170	5500-1270	Capillary ST 0.12x170 S/M

Port	Number of Capillary	Connection	ID x L [mm]	P/N	Description
5	6a	transfer capillary to MHC Valve (OUT), deck B	0.12 x 170	5500-1270	Capillary ST 0.12x170 S/M
6	7	to ^{2}D column	0.12 x 400	5500-1251	Capillary ST 0.12x400 SL/SL
7	9	from ^{2}D pump	0.17 x 280	5067-4608	Capillary ST 0.17x280 SX/S
8	6a	transfer capillary to MHC Valve (IN), deck A	0.12 x 170	5500-1270	Capillary ST 0.12x170 S/M

Connecting the 2D-LC Valve, ASM (G4243A)

In contrast to the standard 2D-LC Valve (G4236A) Agilent recommends using a counter-current configuration for the ASM 2D-LC Valve (G4243A) when working in ASM mode. This section describes the setup for a counter-current configuration of the ASM Valve. For the concurrent setup, please refer to concurrent configuration of the ASM 2D-LC Valve in the 2D-LC Software. You find the Valve topology configuration screen in OpenLab ChemStation under Instrument > 2D-LC Configuration or in OpenLab CDS and MassHunter under 2D-LC Valve Topologies in the LC Driver Online help.

The installation of a 2D-LC system depends on which modules you are using for which 2D-LC mode and is described above. The connection scheme is displayed in the graphical user interface of the 2D-LC Configuration as 2D-LC Valve Topologies:

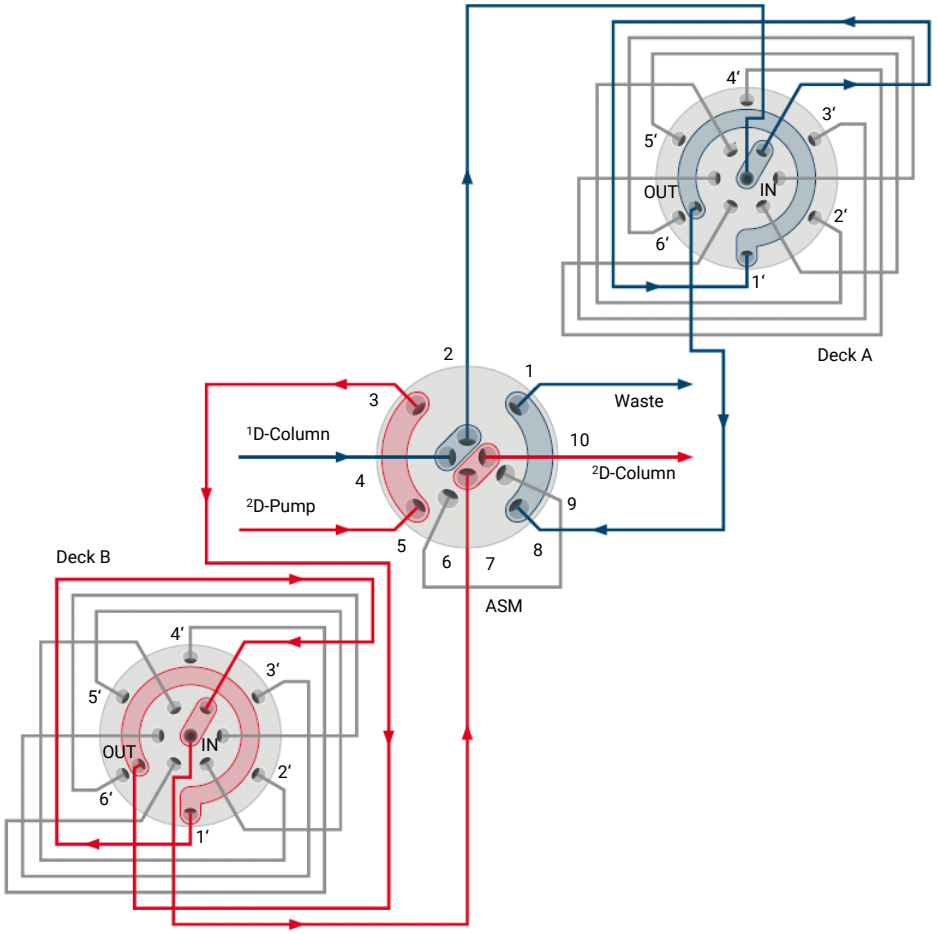


Figure 32: Schematic representation of the ASM 2D-LC Valve (G4243A) in countercurrent flow.

NOTE Against the example shown in the figure above, for 1200 bar MHC Valves that have a different symmetry, the connection is OUT/IN.

Port	Number of Capillary	Connection	ID x L [mm]	P/N	Description
1	11	waste line	0.7 x self-cut	0890-1713	Tubing-flexible 0.8/1.61mm PTFE WT (delivered with UV detector)
2	6b	transfer capillary to MHC Valve (IN), deck A	0.12 x 170	5500-1376	Capillary ST 0.12x170 M/M

Port	Number of Capillary	Connection	ID x L [mm]	P/N	Description
3	6b	transfer capillary from MHC Valve (OUT), deck B	0.12 x 170	5500-1376	Capillary ST 0.12x170 M/M
4	5 F3	from pressure release kit; from ^{1}D column, ^{1}D detector	0.17 x 105 0.12 x 500	5500-1240 5500-1157	Capillary ST 0.17x105 SL/SL Capillary ST 0.12x500 SL/S
5	9	from ^{2}D pump	0.17 x 280	5067-4608	Capillary ST 0.17x280 SX/S
6	ASM1-4	outlet to ASM capillary	0.12 x L		see list below
7	6b	transfer capillary to MHC Valve (IN), deck B	0.12 x 170	5500-1376	Capillary ST 0.12x170 M/M
8	6b	transfer capillary from MHC Valve (OUT), deck A	0.12 x 170	5500-1376	Capillary ST 0.12x170 M/M
9	ASM1-4	inlet from ASM capillary	0.12 x L		see list below
10	7	to ^{2}D column	0.12 x 400	5500-1251	Capillary ST 0.12x400 SL/SL

Which ASM capillary shall be used depends on the ASM factor, which is optimum for your application. You may choose from following capillaries:

Table 11: Available ASM Capillaries and properties

Capillary p/n	Length (mm)	Inner diameter (mm)	Volume (µl)	ASM factor	Split ratio (loop:ASM)
5500-1300	85	0.12	0.96	5	1:4
5500-1301	170	0.12	1.9	3	1:2
5500-1302	340	0.12	3.8	2	1:1
5500-1303	680	0.12	7.7	1.5	1:0.5

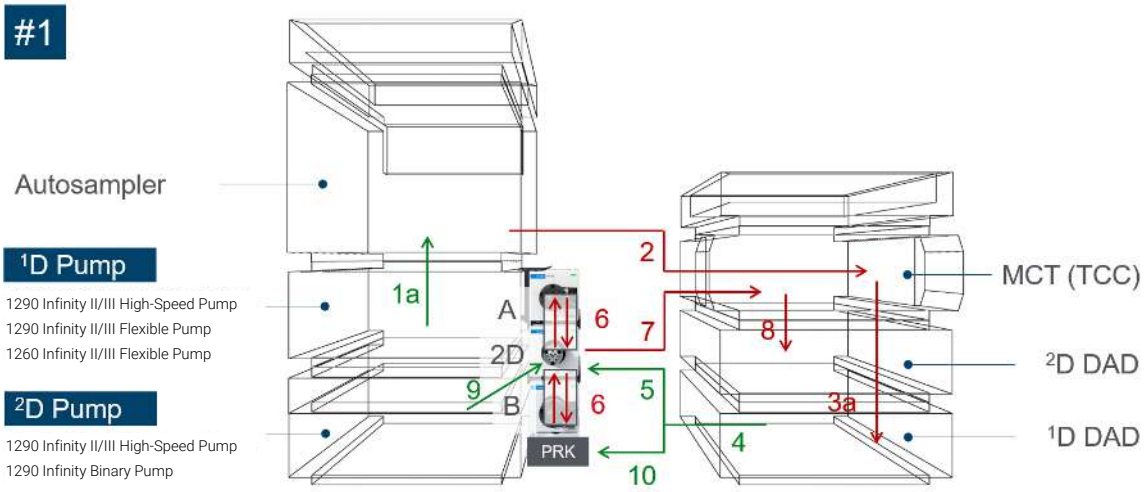


Figure 33: Stack Setup #1. Recommended setup if both pumps are Infinity II/III modules or the 2D pump is a 1290 Infinity Binary pump.

Number of Capillary	#	Connection	ID x L [mm]	P/N	Description
1a	1	1D pump (top) to autosampler	0.17 x 400	5500-1245	Capillary ST 0.17x400 SI/SI
2	1	Autosampler to 1D column (in MCT)	0.12 x 600	5067-4669	Capillary ST 0.12x600 S/SL
3a	1	1D column to 1D DAD	0.12 x 400	5500-1251	Capillary ST 0.12x400 SL/SL
4	1	1D DAD to T-piece of PRK	0.17 x 400	5500-1245	Capillary ST 0.17x400 SI/SI
5	1	T-piece of PRK to Standard 2D-LC Valve (Port 3) / ASM Valve (Port 4)	0.17 x 105	5500-1240	Capillary ST 0.17x105 SL/SL
6a	4	2D-LC Valve (1) - Deck (IN) – Deck (Out) - 2D-LC Valve (8) 2D-LC Valve (5) - Deck (IN) – Deck (Out) - 2D-LC Valve (4)	0.12 x 170	5500-1270	Capillary ST 0.12x170 S/M
6b	4	ASM Valve (7) - Deck (IN) – Deck (Out) - ASM Valve (3) ASM Valve (2) - Deck (IN) – Deck (Out) - ASM Valve (8)	0.12 x 170	5500-1376	Capillary ST 0.12x170 M/M (delivered with 2D-LC Valve Kit, ASM)
7	1	2D-LC valve (6) / ASM valve (10) to 2D column (in MCT)	0.12 x 400	5500-1251	Capillary ST 0.12x400 SL/SL
8	1	2D column (in MCT) to 2D DAD	0.12 x 280	5067-4651	Capillary ST 0.12x280 SL/SX
9	1	2D pump to 2D-LC Valve (7) / ASM Valve (5)	0.17 x 280	5067-4608	Capillary ST 0.17x280 SX/S

Number of Capillary	#	Connection	ID x L [mm]	P/N	Description
10	1	T-piece of PRK to damper capillary	0.17 x 150	5500-1227	Capillary ST 0.17x150 SL/SL
11	1	waste line	0.7 x self-cut	0890-1713	Tubing-flexible 0.8/1.61mm PTFE WT (delivered with UV detector)

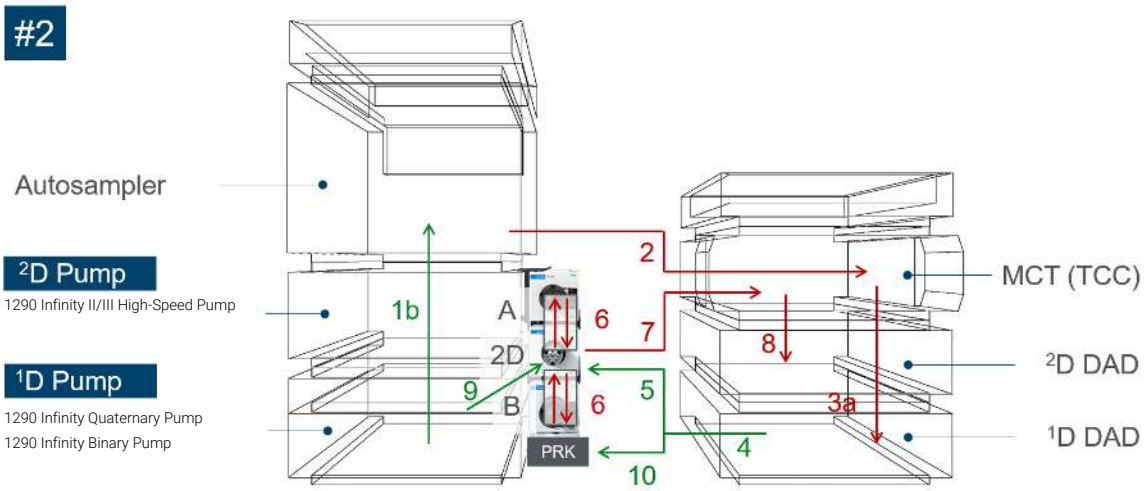


Figure 34: Stack Setup #2. Recommended setup if the [1]D pump is a 1290 Infinity Binary Pump or a 1290 Infinity Quaternary Pump.

Number of Capillary	#	Connection	ID x L [mm]	P/N	Description
1b	1	[1]D pump (bottom) to sampler	0.17 x 600	5067-4670	Capillary ST 0.17x600 S/SH
2	1	Autosampler to [1]D column (in MCT)	0.12 x 600	5067-4669	Capillary ST 0.12x600 S/SL
3a	1	[1]D column to [1]D DAD	0.12 x 400	5500-1251	Capillary ST 0.12x400 SL/SL
4	1	[1]D DAD to T-piece of PRK	0.17 x 400	5500-1245	Capillary ST 0.17x400 SI/SI
5	1	T-piece of PRK to Standard 2D-LC Valve (Port 3) / ASM Valve (Port 4)	0.17 x 105	5500-1240	Capillary ST 0.17x105 SL/SL
6a	4	2D-LC Valve (1) - Deck (IN) – Deck (Out) - 2D-LC Valve (8) 2D-LC Valve (5) - Deck (IN) – Deck (Out) - 2D-LC Valve (4)	0.12 x 170	5500-1270	Capillary ST 0.12x170 S/M
6b	4	ASM Valve (7) - Deck (IN) – Deck (Out) - ASM Valve (3) ASM Valve (2) - Deck (IN) – Deck (Out) - ASM Valve (8)	0.12 x 170	5500-1376	Capillary ST 0.12x170 M/M (delivered with 2D-LC Valve Kit, ASM)

Number of Capillary	#	Connection	ID x L [mm]	P/N	Description
7	1	2D-LC valve (6) / ASM valve (10) to ^{2}D column (in MCT)	0.12 x 400	5500-1251	Capillary ST 0.12x400 SL/SL
8	1	^{2}D column (in MCT) to ^{2}D DAD	0.12 x 280	5067-4651	Capillary ST 0.12x280 SL/SX
9	1	^{2}D pump to 2D-LC Valve (7) / ASM Valve (5)	0.17 x 280	5067-4608	Capillary ST 0.17x280 SX/S
10	1	T-piece of PRK to damper capillary	0.17 x 150	5500-1227	Capillary ST 0.17x150 SL/SL
11	1	waste line	0.7 x self-cut	0890-1713	Tubing-flexible 0.8/1.61mm PTFE WT (delivered with UV detector)

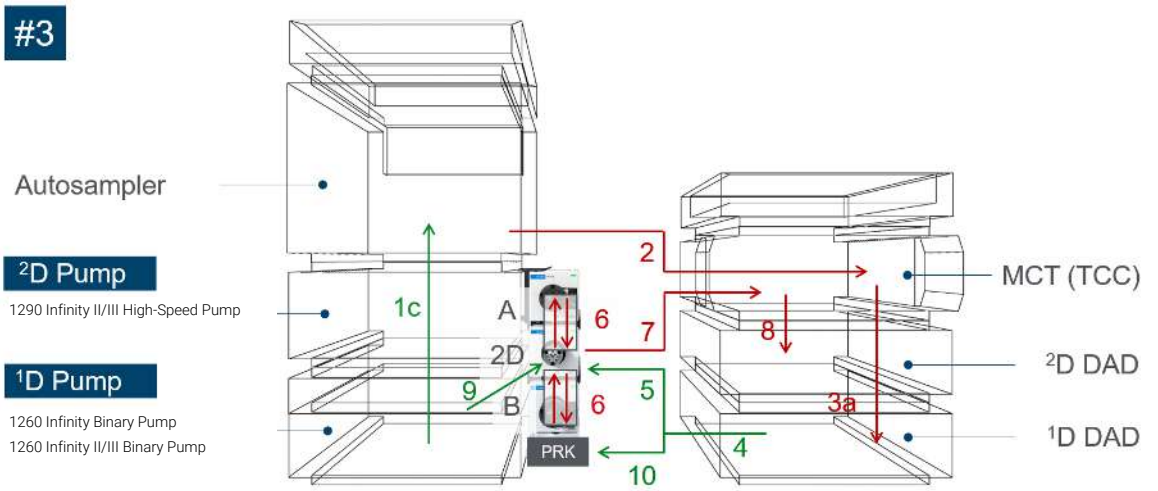


Figure 35: Stack Setup #3. Recommended setup if the ^{1}D pump is a 1260 Infinity or 1260 Infinity II/III Binary Pump.

Number of Capillary	#	Connection	ID x L [mm]	P/N	Description
1c	1	^{1}D pump (bottom) to sampler	0.17 x 900	5500-1217	Capillary ST 0.17x900 SI/SX
2	1	Autosampler to ^{1}D column (in MCT)	0.12 x 600	5067-4669	Capillary ST 0.12x600 S/SL
3a	1	^{1}D column to ^{1}D DAD	0.12 x 400	5500-1251	Capillary ST 0.12x400 SL/SL
4	1	^{1}D DAD to T-piece of PRK	0.17 x 400	5500-1245	Capillary ST 0.17x400 SI/SI
5	1	T-piece of PRK to Standard 2D-LC Valve (Port 3) / ASM Valve (Port 4)	0.17 x 105	5500-1240	Capillary ST 0.17x105 SL/SL

Number of Capillary	#	Connection	ID x L [mm]	P/N	Description
6a	4	2D-LC Valve (1) - Deck (IN) – Deck (Out) - 2D-LC Valve (8) 2D-LC Valve (5) - Deck (IN) – Deck (Out) - 2D-LC Valve (4)	0.12 x 170	5500-1270	Capillary ST 0.12x170 S/M
6b	4	ASM Valve (7) - Deck (IN) – Deck (Out) - ASM Valve (3) ASM Valve (2) - Deck (IN) – Deck (Out) - ASM Valve (8)	0.12 x 170	5500-1376	Capillary ST 0.12x170 M/M (delivered with 2D-LC Valve Kit, ASM)
7	1	2D-LC valve (6) / ASM valve (10) to ^{2}D column (in MCT)	0.12 x 400	5500-1251	Capillary ST 0.12x400 SL/SL
8	1	^{2}D column (in MCT) to ^{2}D DAD	0.12 x 280	5067-4651	Capillary ST 0.12x280 SL/SX
9	1	^{2}D pump to 2D-LC Valve (7) / ASM Valve (5)	0.17 x 280	5067-4608	Capillary ST 0.17x280 SX/S
10	1	T-piece of PRK to damper capillary	0.17 x 150	5500-1227	Capillary ST 0.17x150 SL/SL
11	1	waste line	0.7 x self-cut	0890-1713	Tubing-flexible 0.8/1.61mm PTFE WT (delivered with UV detector)

Alternative instrument setups for additional functionality

The standard stack setups can be upgraded with additional valves to add additional functionality. Table 10 on page 62 gives an overview of all supported modifications of a standard 2D-LC instrument. At a time, only one modification is recommended to ensure correct operation of the instrument. The standard stack setup uses one column compartment that hosts both the ^{1}D and ^{2}D column.

Table 12: List up supported modifications of a standard 2D-LC instrument configuration.

Alternative column compartment concepts		Comment	Page
A	^{1}D MCT/TCC hosts column switching valve	If a 6-position/14-port or 8-position/18-port InfinityLab Quick Change Valve is used, additional two adapters necessary (2xG1316-87326, must be purchased separately)	See Figure 36 on page 72
B	Setups that contain separate ^{1}D and ^{2}D MCTs/TCCs		See Figure 37 on page 73
C	Setups in which the ^{1}D column is hosted in an Integrated Column Compartment (ICC)	Longer capillary (5500-1170) for Quick Connect Fitting at column inlet or new 0.12x280mm Quick Connect Fitting assembly (5067-5960) necessary (must be purchased separately).	See Figure 38 on page 74

Alternative column compartment concepts		Comment	Page
D	Setup with a MS diverter valve		See Figure 39 on page 75
E	Setup of a ^{1}D/^{2}D Switching Valve	If a ^{1}D and ^{2}D detector is used; not supported with modifications A-C	See Figure 40 on page 76
F	^{1}D/^{2}D Switching Valve w/o ^{1}D detector	For setups that do not have a ^{1}D detector, e.g. for certain LCxLC setups or setups with a QQQ mass spectrometer as a ^{2}D detector; not supported with modifications A-C	See Figure 41 on page 77
G	Single Heart-Cutting Configuration as Single Sample Loop Setup	For this setup port 4 and port 5 of the 2D-LC Standard must be used to connect the single loop while the bypass capillary is installed at the other position (Port 1 and 8) (for instance see application G4245A ProtA-SEC Kit).	See Figure 42 on page 78

A

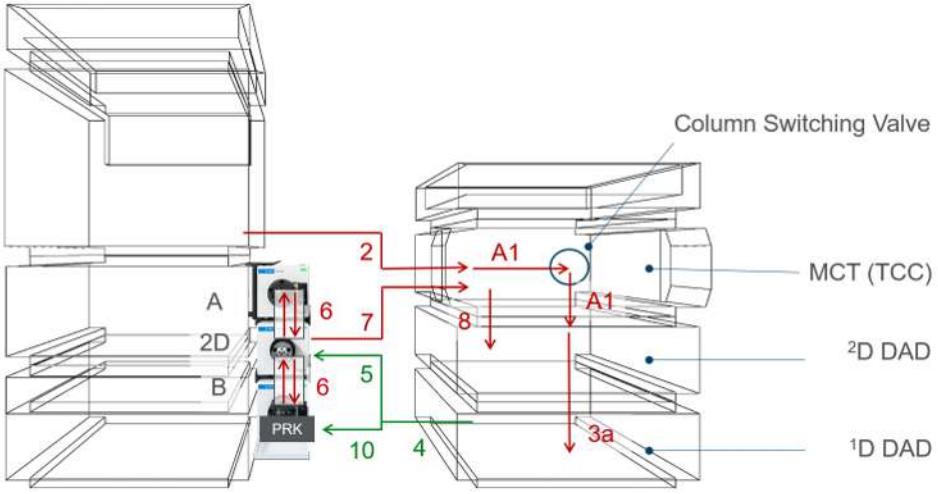


Figure 36: Setup A. Recommended setup if a column switching valve (for example 6-position/14-port InfinityLab Quick-Change Valve) is used. For a InfinityLab 2-position/6-port Quick-Change Valve, adapters A1 are not necessary.

Number of Capillary	#	Connection	ID x L [mm]	P/N	Description
A1	2	Adapter: capillary 2 to column switching valve, (Port IN) / Adapter column switching valve (Port OUT) to capillary 3a	0.12 x 75	G1316-87326	SST Capillary 0.12x75mm, f/m, ns 0.8 (must be purchased separately)

For all other capillaries / connections, please refer to Figure 33 on page 68, Figure 34 on page 69, and Figure 35 on page 70.

NOTE Adapters to and from the column switching valve are only necessary if a 6-position/14-port InfinityLab Quick-Change Valve or a for example 8-position/18-port InfinityLab Quick-Change Valve is used.

B

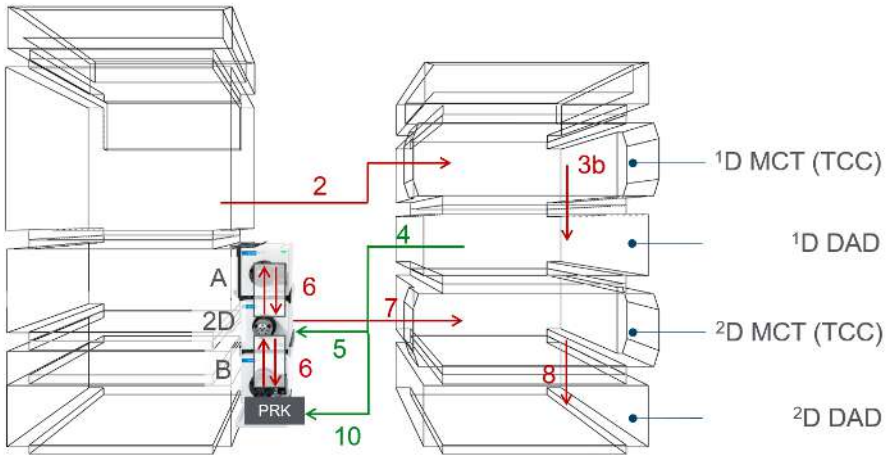


Figure 37: Setup B. Recommended setup if the instrument contains separate MCTs/ TCCs for ^{1}D and ^{2}D columns.

Number of Capillary	#	Connection	ID x L [mm]	P/N	Description
3b	1	^{1}D column to ^{1}D DAD	0.12 x 280	5067-4651	Capillary ST 0.12x280 SL/ SX
8	1	^{2}D column (in ^{2}D MCT) to ^{2}D DAD	0.12 x 280	5067-4651	Capillary ST 0.12x280 SL/ SX (part of 2D-LC capillary kit)

For all other capillaries / connections, please refer to Figure 33 on page 68, Figure 34 on page 69, and Figure 35 on page 70.

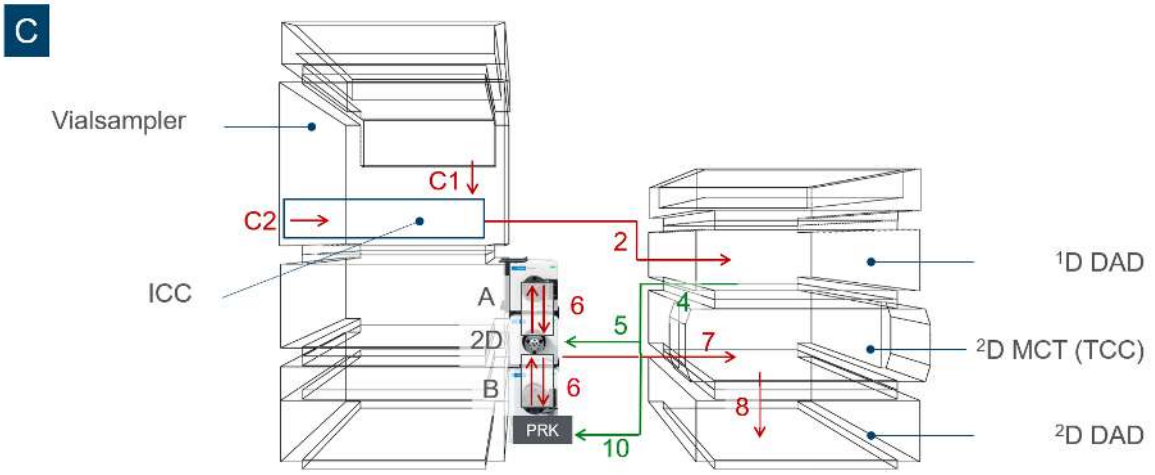


Figure 38: Setup C. Recommended setup if [1]D column is hosted in an Integrated Column Compartment (ICC).

Number of Capillary	#	Connection	ID x L [mm]	P/N	Description
C1	1	Injection Valve to ICC	0.12 x 105	5500-1238	Capillary ST 0.12x105 SL/ SL (provided with ICC)
C2	1	Heat exchanger out to column (InfinityLab Quick Connect Fitting)	0.12 x 280	5500-1170	Capillary ST 0.12x280 (must be purchased separately)
8	1	[2]D column (in [2]D MCT) to [2]D DAD	0.12 x 280	5067-4651	Capillary ST 0.12x280 SL/ SX (part of 2D-LC capillary kit)

For all other capillaries / connections, please refer to Figure 33 on page 68, Figure 34 on page 69, and Figure 35 on page 70.

The driver-based 2D-LC Solution allows only certain valves to be configured as diverter valves which can be used for example as an effective desalting tool.

A list of supported valves can be found in Table 10 on page 62

More information is available in the following sections:

- Method Parameters on page 152
- Run the System on page 228

Qty.	p/n	Description
1	G4231A	2pos/6port Valve head 600 bar

Qty.	p/n	Description
1	G4231C	2pos/6port valve head, 1300 bar
1	G4232C	2pos/10port valve head, 800 bar
1	G4232D	2pos/10port valve head, 1300 bar

D

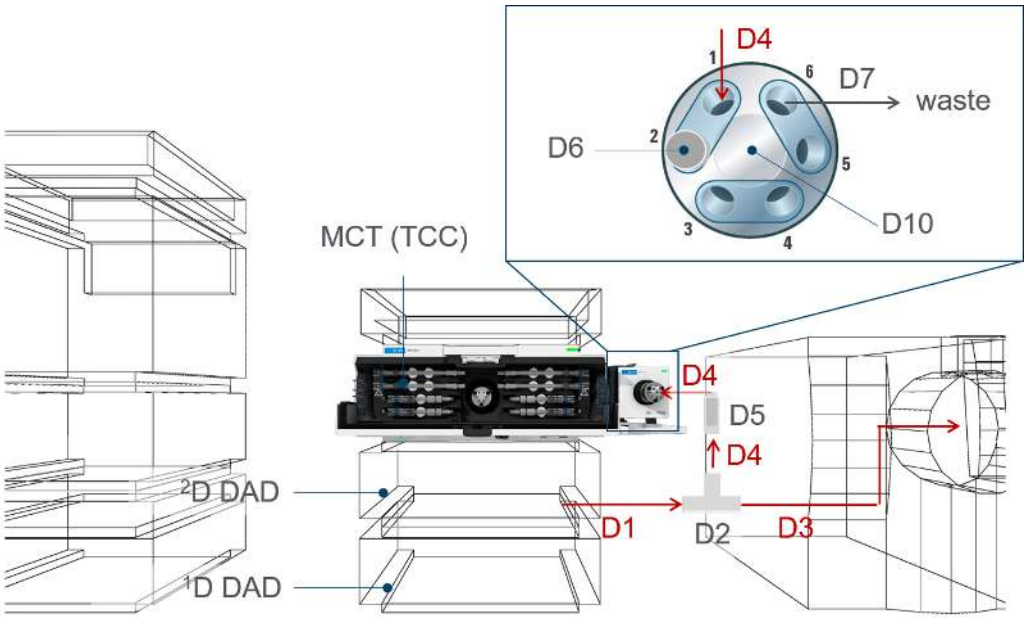


Figure 39: Setup D. Recommended setup of a MS diverter valve.

Number of Capillary	#	Connection	ID x L [mm]	P/N	Description
D1	1	Capillary from ^{2}D detector to T-piece	0.12 x 400	5067-4606	Capillary ST 0.12x400 S/SH
D2	1	T-piece		0100-0969	1/16in Tee, SST, Low Dead Volume
D3	1	Capillary from MS to T-piece (self cut)	0.12 x 400	0890-1915	Capillary PEEK, 0.12x1250
D4	2	T-piece to pressure relief valve; pressure relief valve to diverter valve	0.3 x 80	5500-1228	Capillary ST 0.3x80 SL/SL
D5	1	Pressure relief valve		G4212-60022	Pressure relief valve
D6	1	blank nut		01080-83202	Blanking Nut 1/16 in SST
D7	1	diverter valve to waste		5062-2462	Tubing PTFE 0.7 mm x 5m

Number of Capillary	#	Connection	ID x L [mm]	P/N	Description
D8	1	peak fittings		5063-6591	Fitting-Fingertight PEEK for 1/16-in
D9	1	Valve holder for Valve drive to attach to MCT		5067-6138	Valve Holder Kit Right-IF-II-G
D10	1	Diverter Valve		G4231A G4231C G4232A G4232C	2pos/6port, 800bar 2pos/6port, 1300bar 2pos/10port, 800bar 2pos/10port, 1300bar

For all other capillaries / connections, please refer to Figure 33 on page 68, Figure 34 on page 69, and Figure 35 on page 70.

The ^{1}D/^{2}D switching valve offers the possibility to exclude the ^{2}D flow path of the instrument to run both ^{1}D and ^{2}D experiments which is useful for example if one mass spectrometer is used for both ^{1}D and ^{2}D experiments. Two basic setups are supported (setup E and F). The recommended setups for a ^{1}D/^{2}D Switching valves do not support the use of ICC column compartments, column switching valves or the use of separate ^{1}D and ^{2}D MCTs/TCCs! To run 1D experiments, the ^{2}D mode must be disabled. This must be done in the UI of the 2D-LC Method Editor, see Off on page 156.

E

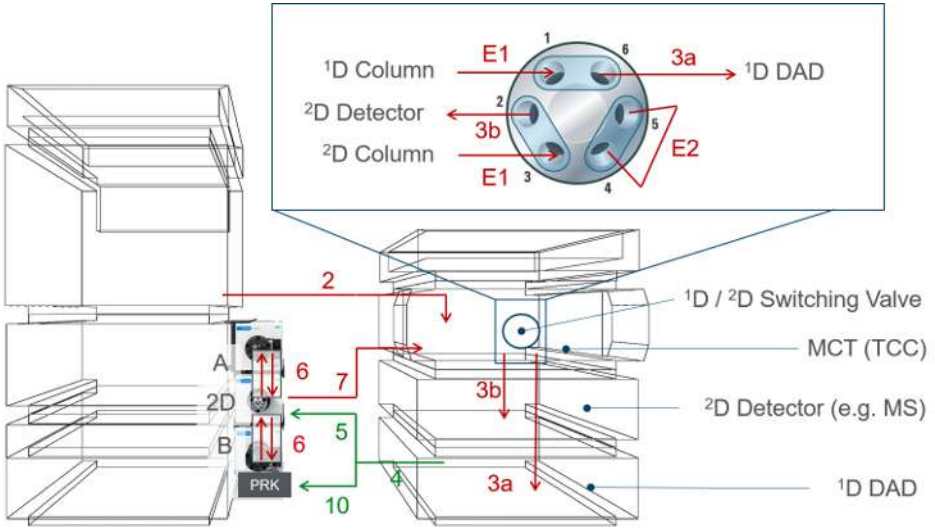


Figure 40: Setup E. Recommended setup for the ^{1}D/^{2}D switching valve.

Number of Capillary	#	Connection	ID x L [mm]	P/N	Description
3a	1	MCT / TCC to ^{1}D DAD	0.12 x 400	5500-1251	Capillary ST 0.12x400 SL/ SL
8	1	^{1}D MCT / TCC to ^{1}D DAD	0.12 x 280	5067-4651	Capillary ST 0.12x280 SL/ SX
E1	2	^{1}D column to ^{1}D/^{2}D Switching Valve (1); ^{2}D column to ^{1}D/^{2}D Switching Valve (3)	0.12 x 120	5067-4652	Capillary ST 0.12x120 SX/ SX
E2	1	Connection capillary ^{1}D/^{2}D Switching Valve (4) to (5)	0.12 x 90	5067-4649	Capillary ST 0.12x90 SX/S
For all other capillaries / connections, please refer to Figure 33 on page 68, Figure 34 on page 69, and Figure 35 on page 70.					

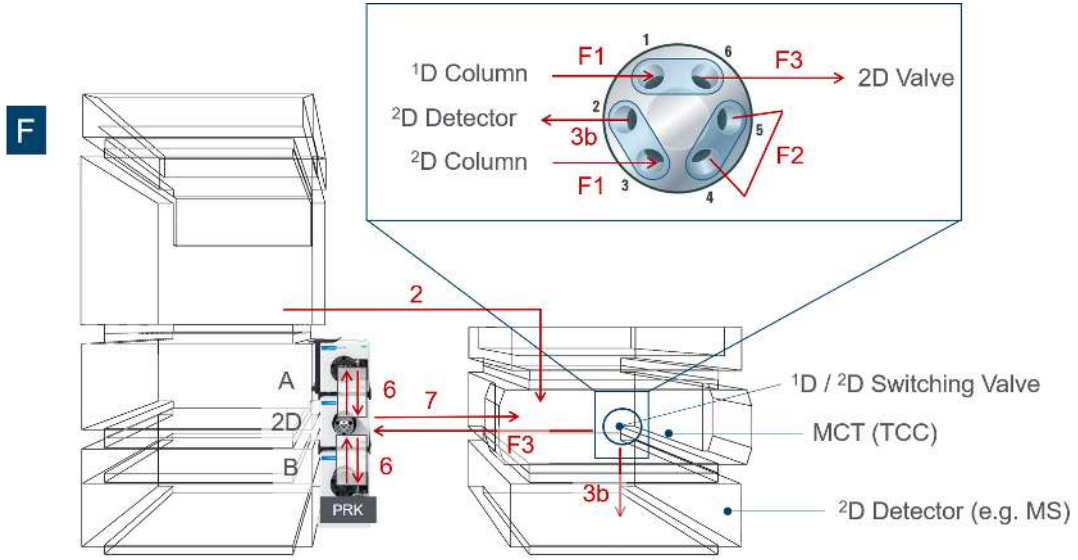


Figure 41: Setup F. Recommended setup for the ^{1}D/^{2}D switching valve without ^{1}D detector.

Number of Capillary	#	Connection	ID x L [mm]	P/N	Description
3b	1	^{1}D/^{2}D Switching Valve (2) to ^{2}D DAD	0.12 x 280	5067-4651	Capillary ST 0.12x280 SL/ SX
F1	2	^{1}D column to ^{1}D/^{2}D Switching Valve (1); ^{2}D column to ^{1}D/^{2}D switching valve (3)	0.12 x 120	5067-4652	Capillary ST 0.12x120 SX/ SX
F2	1	Connection ^{1}D/^{2}D switching valve ports (4) to (5)	0.12 x 90	5067-4649	Capillary ST 0.12x90 SX/S

Number of Capillary	#	Connection	ID x L [mm]	P/N	Description
F3	1	MCT/TCC to 2D-LC valve (6) / ASM valve (4)	0.12 x 500	5500-1157	Capillary ST 0.12x500 SL/S

For all other capillaries / connections, please refer to Figure 33 on page 68, Figure 34 on page 69, and Figure 35 on page 70.

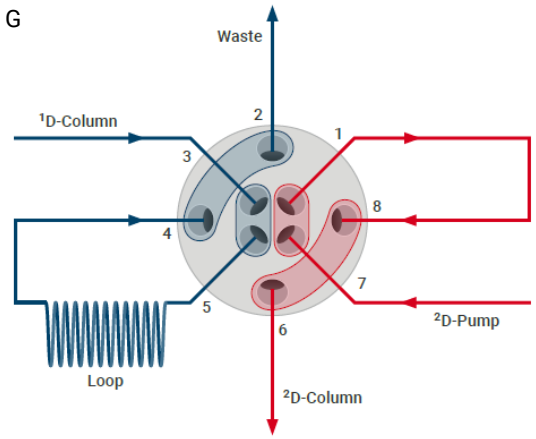


Figure 42: Setup G. Single Heart-Cutting Configuration as Single Sample Loop Setup

Number of Capillary	#	Connection	ID x L [mm]	P/N	Description
1	1	Bypass capillary (OUT)	0.12 x 105	5500-1238	Capillary, ST 0.12x105 SL/SL
2	1	Waste line	0.7 x self-cut	0890-1713	Tubing-flexible 0.8/1.61mm PTFE WT (delivered with UV detector)
3	1	From pressure release kit; from ^{1}D column, ^{1}D detector	 0.17 x 105 0.12 x 500	 5500-1240 5500-1157	 Capillary ST 0.17x105 SL/SL Capillary ST 0.12x500 SL/S
4		Sample Loop (IN)		5004-0036	180 µL Loop 2D-LC as an example
5		Sample Loop (OUT)		5004-0036	180 µL Loop 2D-LC as an example
6	1	To ^{2}D column	0.12 x 400	5500-1251	Capillary ST 0.12x400 SL/SL
7	1	From ^{2}D pump	0.17 x 280	5067-4608	Capillary ST 0.17x280 SX/S
8		Bypass capillary (IN)	0.12 x 105	5500-1238	Capillary, ST 0.12x105 SL/SL

For all other capillaries / connections, see Figure 33 on page 68, Figure 34 on page 69, and Figure 35 on page 70.

NOTE

If the dual-loop setup has been selected in the software configuration (see Configure the 2D-LC Cluster on page 119), install mirror-inverted, the sample loop at port 1 and 8 and the bypass capillary at position 4 and 5.

Installing the Pressure Release Kit

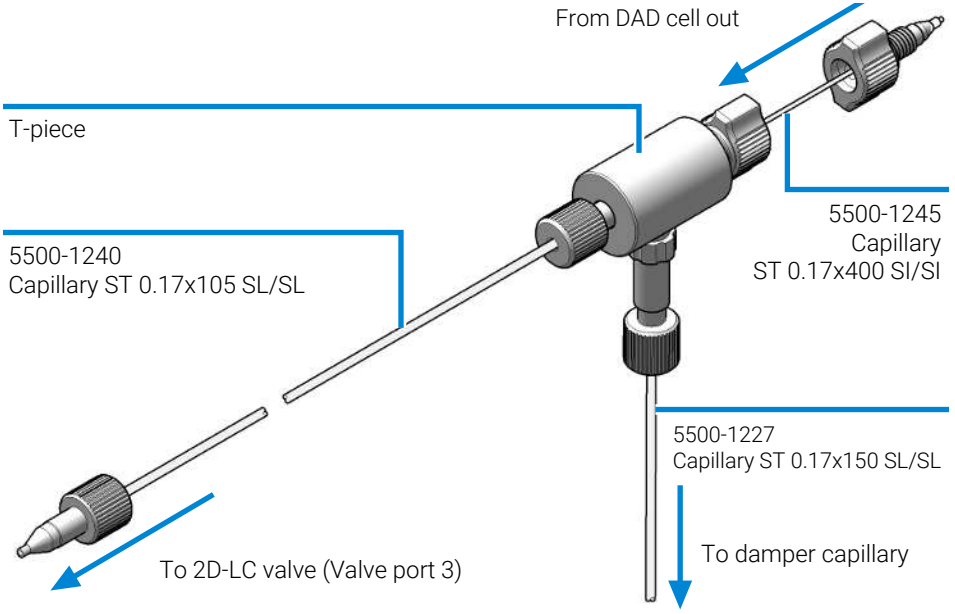
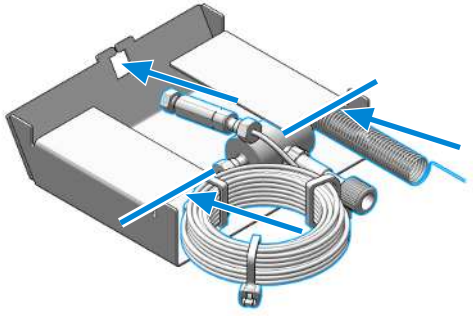


Figure 43: Connections to the pressure release kit

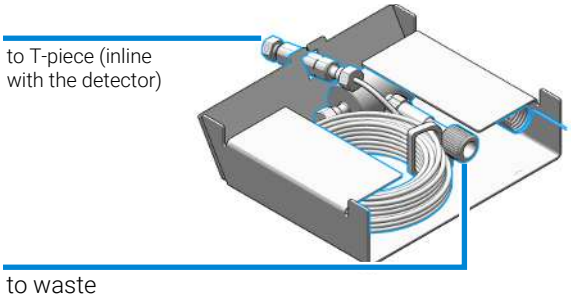
Parts required

Qty.	p/n	Description
1	G4236-60010	2D-LC Pressure Release Kit

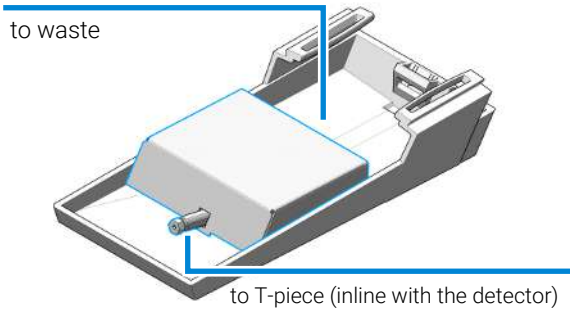
1 Push the pressure release valve assembly in the frame.



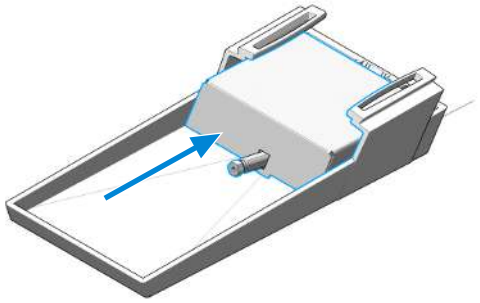
2 Take care for the correct orientation.



3 Insert the pressure release assembly to the leak tray, orientation as shown.



4 Push the pressure release assembly in the correct position.



5 Connect with the T-piece, see Figure 43 on page 80.

Install the Valve Head and Connecting Capillaries

For instructions on how to install the valve head and connecting capillaries, see Replace Valve Heads (G1170A) on page 368.

Hardware Installation of the Bio 2D-LC System

Delivery Checklist

For biocompatible modules use bio / biocompatible parts only!

Do not mix with bio-inert parts.

The InfinityLab Bio 2D-LC ASM Valve kit (G5643B) contains the following parts:

Qty.	p/n	Description
1	5005-0078	Agilent InfinityLab Bio 2D-LC ASM Valve
1	5190-6895	2D-LC starter sample, 1 x 2 mL
2	G5642-64000	Multiple Heart-Cutting Valve
1	699968-301	Poroshell 120 Bonus-RP, 3.0 x 50 mm, 2.7 µm
1	G4236-64000	2D-LC Easy Start USB Media Kit
1	5005-0077	InfinityLab Bio 2D-LC Capillary Kit
1	G2453-85060	Formic Acid-Reagent Grade 5 mL (5 cc)
1	685775-902	InfinityLab Poroshell 120 SB-C18, 2.1 x 100 mm, 2.7 µm
1	G1680-63721	Network LAN Switch
1		Regional power cord

The InfinityLab Bio 2D-LC Capillary Kit (5005-0077) contains the following parts:

Qty.	p/n	Description
3	5500-1603	Quick Turn Capillary MP35N 0.17 mm x 400 mm
1	5004-0031	Capillary MP35N 0.12 mm x 600 mm
2	G7116-60071	Quick Connect Bio Heat Exchanger Standard Flow
2	5500-1578	Quick Connect Capillary MP35N 0.12 mm x 105 mm
2	5500-1597	Quick Turn Capillary MP35N 0.12 mm x 400 mm
1	5500-1599	Quick Turn Capillary MP35N 0.17 mm x 105 mm
1	5500-1600	Quick Turn Capillary MP35N 0.17 mm x 150 mm

Qty.	p/n	Description
1	5500-1596	Quick Turn Capillary MP35N 0.12 mm x 280 mm
2	5067-5965	InfinityLab Quick Connect LC fitting
20	5067-5966	InfinityLab Quick Turn Fitting
1	0890-1713	Tubing, PTFE, ID/OD 0.8 /1.6 mm
1	5063-6591	PEEK Fittings 10/PK

The Bio Compatible MHC Loop Assembly SST (G5642-64000) contains the following parts:

p/n	Description
5043-0269	Adapter-profile for G1170A
5067-4273	6-column selector valve head, 1300 bar
5004-0027	Capillary MP35N 0.35 mm x 420 mm M/M 40 µL (6x) Pre-installed on 6 column selector

NOTE Depending on the set up of you instrument, extra parts and capillaries might be required for installation. Those parts are ordered separately or are shipped with other components. Their origin as well as their function is described in the instrument setup section below or in the 2D-LC User manual or in the Bio LC device manuals.

Bio Materials

For the Bio LC System, Agilent Technologies uses highest-quality materials in the flow path (also referred to as wetted parts). Life scientists prefer these materials, as they are known for optimum inertness to biological samples and ensure best compatibility with common samples and solvents over a wide pH range. To enable chromatography at very high pressures, while maintaining inertness the metal alloy MP35N is used instead of stainless steel throughout the system.

The MP35N is a nonmagnetic, nickel-cobalt-chromium-molybdenum alloy with an excellent resistance to sulfation, oxidation, saline solutions, and most mineral acids. Its superior properties ensure reliable performance, even under UHPLC conditions.

Bio Part Identification

CAUTION

BIO vs. BIO INERT

Bio-inert parts are made of PEEK or other low pressure rated materials and cannot withstand high pressure above 600 bar.

Bio-inert parts are *not compatible* with 1290 Bio LC modules.

- For 1290 Bio LC modules, use bio/biocompatible parts only.
- For bio-inert modules, use bio-inert parts only.
- Do not mix parts between 1260 Bio-Inert LC modules and 1290 Bio LC modules.

NOTE

The installation of stainless steel-cladded PEEK capillaries (bio-inert) requires a special handling. Please read the Technical Note G5611-90120 (Installation of Stainless Steel Cladded PEEK Capillaries.) for further and detailed description.

Important Hints for the Use of Bio Capillaries in a 1290 Bio LC System

CAUTION

HNO_3 based procedures, and/or stainless steel in the flow path.

Damage of parts.

Metal ions may be introduced to the originally iron-free flow path.

- Do not us HNO_3-based procedures for the 1290 Bio LC System.
- Do not install mixed systems including biocompatible and regular stainless steel modules, parts, or capillaries.

NOTE

The Technote *Best Practices for Using an Agilent LC System Technical Note (InfinityLab-BestPractice-en-SD-29000194.pdf, SD-29000194)* contains recommendations for 1290 Bio modules like installation, operation, and maintenance procedures.

Maintenance intervals of the bio valve may vary depending on the operation mode and the different solvents used, such as solvents with high buffer concentrations.

NOTE

To ensure optimum biocompatibility of your 1290 Bio LC System:

— Do not include non-Bio standard modules or parts to the flow path

— Do not use any parts that are not labeled as Agilent *Bio*

⇨ For solvent compatibility of bio, biocompatible, and bio-inert materials, see *General Information about Solvent/Material Compatibility* in the Bio LC user manuals.

NOTE

Do not use stainless steel capillaries in the 1290 Bio LC System. Watch out for orange stripe on the PTFE tubing of the capillary.

To avoid salt precipitation and blockages:

— Do not exceed or approach the solubility limit of buffer salt when prepare solvents

— Do not use > 50 mM buffer salt with high (> 60 %) acetonitrile concentrations

Important Notice on Fittings

Poroshell and AdvanceBio PEEK-lined columns

- Care must be taken to avoid damage to PEEK-lined columns during installation. Combined compression and rotation may cause internal damage. Fittings without ferrules (such as PEEK finger-tight fittings) are not recommended.
- Either use Agilent stainless steel cladded PEEK capillaries (1260 bio-inert solution) or MP35N capillaries with Quick Turn or Quick Connect fittings (1290 biocompatible solution).
- To choose the best fitting and capillary for bio-inert instrument setup

  www.agilent.com/chem/bioinertfittings
- To choose the best fitting and capillary for stainless steel system

  www.agilent.com/chem/fittings

Options

NOTE

The 1290 Infinity III Bio 2D-LC System must contain an Agilent Infinity III Bio High-Speed Pump (G7132A) as ^{2}D pump.

This is necessary to achieve the following:

- Enable 2D-LC functionality
- Run fast gradients on the ^{2}D column

Module identification: The module identifier (e.g. G7117A) can be found on the lower right side of the module front cover.

Table 13: Overview of recommended bio hardware configurations

Function	Functional Element	Part Number	Module	Comment
^{1}D	Pump	G7131A	1290 Bio Flexible Pump	
		G7131C	1260 Bio Flexible Pump	
		G7132A	1290 Bio High-Speed Pump	
		G5654A	1260 Bio-inert Quaternary Pump	
	Sampler	G7137A	1290 Bio Multisampler	
		G5668A	1260 Bio-inert Multisampler	
	Thermostat	G7116A	1260 Multicolumn Thermostat	Column compartments need biocompatible parts in the flow path. The G7116A is limited to use only valves up to 800 bar.
		G7116B	1290 Multicolumn Thermostat	
	Detector	G7165A	1260 Multiple Wavelength Detector	Detectors need biocompatible parts in the flow path. Adjust the ^{1}D flow rate to the flow cell pressure specifications. See also the comment on the Pressure Release Kit.
		G7115A	1260 Diode Array Detector WR	
		G7114A	1260 Variable Wavelength Detector	
		G7114B	1290 Variable Wavelength Detector	
		G7117A	1290 Diode Array Detector FS	
		G7117B	1290 Diode Array Detector	
Interface	Valve drive	G1170A	1290 Valve Drive	

Function	Functional Element	Part Number	Module	Comment
	Bio 2D-LC Valve	G5643B	InfinityLab Bio 2D-LC ASM Valve Kit	For flow path, see Connecting the Bio 2D-LC ASM Valve without MHC on page 96 or Connecting the Bio 2D-LC Valve, ASM with MHC on page 98.
	MHC Valves		InfinityLab Bio Multiple Heart-Cutting Valve	These valves are included in G5643B. Stainless steel valves and biocompatible capillaries.
	Pressure Release Kit (PRK)	G4236-60 010	Pressure Release Kit	Mandatory if a ^{1}D detector is used. The kit prevents pressure pulses and protects detector flow cells!

Function	Functional Element	Part Number	Module	Comment
^{2}D	Pump	G7132A	1290 Bio High-Speed Pump	1290 Bio High-Speed Pump required.
	Column Compartment	G7116A	1260 Multicolumn Thermostat	The second column compartment in the Bio 2D-LC System is recommended for large temperature differences between ^{1}D and ^{2}D. Any of these are supported as well as others or older bio modules. Need biocompatible parts in the flow path. The G7116A is limited to use only valves up to 800 bar.
		G7116B	1290 Multicolumn Thermostat	
	Detector	G7117A	1290 Diode Array Detector FS	Need biocompatible parts in the flow path.
		G7117B	1290 Diode Array Detector	
		G7117C	1260 Diode Array Detector HS	
		G7114A	1260 Variable Wavelength Detector	
		G7114B	1290 Variable Wavelength Detector	
		G7115A	1260 Diode Array Detector WR	
		G7165A	1260 Multiple Wavelength Detector	
		G7121B	1260 Fluorescence Detector Spectra	
			Agilent Single Quadrupole Detector LC/MSD	
			High-End mass spectrometer like TOF/QTOF or TQ	

NOTE: It is possible to connect third party detectors via UIB2 G1390A analog digital converter. But these third party modules have limited features in the CDS.
NOTE: Due to potential tailing, G7117A/B and G4212A/B Flow cells are not recommended for WCX and low salt SEC.
NOTE: To analyze photosensitive samples with UV-detectors (e.g. VWD, DAD WR, or LSS), prefer suitable flow cells and low light intensities. This is especially important for detectors in the first dimension.

Recommendations for Bio 2D-LC System

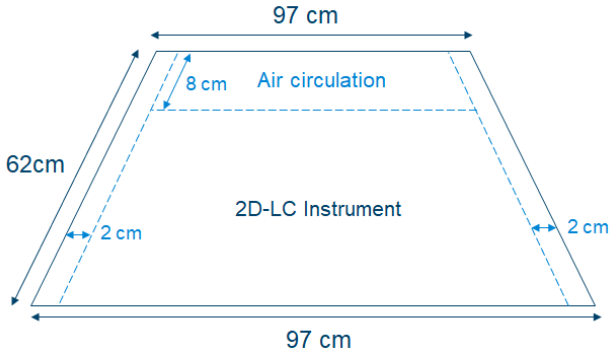
General Information

Bio 2D-LC Systems come in several flavors, still allowing flexible HPLC combination of the Bio LC System and Bio-inert LC. For a biocompatible 2D-LC system, a two-stack configuration is necessary. On the left stack, the order of the modules from bottom to top is: bio pumps for both dimensions, then bio autosampler.

The sampler must be placed on top of the pumps. The recommendation for the right stack consists of two column compartments to be more flexibly in respect to large temperature differences and column sizes and one or two standard UV detectors.

Both stacks offer the possibility to place a solvent cabinet on top.

Table 14: Recommended stack configuration and required bench space

Recommended stack configuration for the Bio 2D-LC System	Bench space requirements of the Bio 2D-LC System
	

NOTE

The dual stack configuration for Bio 2D-LC requires at least 97 x 62 cm (24.4 x 38.2 inches) free, vertical bench space. 2.5 cm (1.0 inches) of space on either side and approximately 8 cm (3.1 inches) in the rear is reserved for air circulation and electric connections.

Installation of the Bio 2D-LC ASM Valve and Optional MHC Decks

Attaching the External Valve Drives

For 2D-LC instruments that comprise at least one bio pump from the 1260 Infinity II/III or 1290 Infinity II/III series, valve drives are attached to this pump with 5067-5685 (Clamp Guide Kit) , while the valve drives are interconnected by 5043-0269 (Adapter-profile) . The Bio 2D-LC valve and the MHC decks are mounted on external valve drives (G1170A).

#	Holders/connectors	Connection	P/N
3	1290 Infinity III Valve Drive (must be purchased separately)	Mounting of Valves	G1170A
1	Clamp Guide Kit (delivered with G1170A)	Top valve to pump	5067-5685
2	Adapter-profile (delivered with MHC Decks)	between G1170A drives	5043-0269

For an SHC configuration, the Bio 2D-LC ASM valve (G5643B) is attached to the upper pump of the stack. In an MHC configuration, the upper MHC deck is attached to the upper pump.

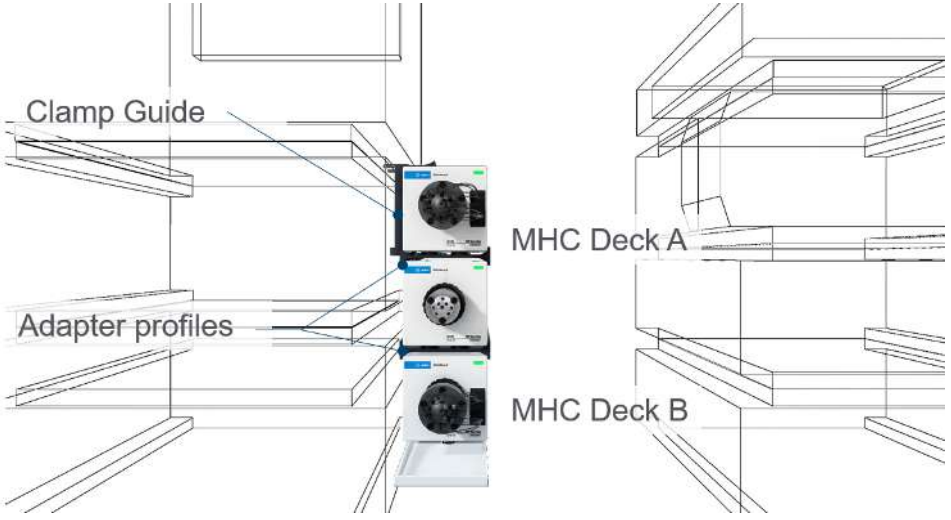


Figure 44: Schematic of the installation and attachments of the Bio 2D-LC valve and optionally the MHC decks

1 Mount the clamp guide on the right side of the pump: Markings in the form of round dips are on the body housing. Make a small hole with a peaked screw driver and tighten the clamp guide with the three self-cutting tapping screws.

2 Mount the valve heads on the G1170A external valve drives.

3 Clamp the first external valve drive with the MHC valve on top.

4 Attach the adapter-profile on each of the other external valve drives and mount them according to the positions shown in Figure 44 on page 91.

5 Mount the leak tray with sensor underneath the lowest external valve drive.

6 Install the pressure release kit, see Installing the Pressure Release Kit on page 104.

Valve Configurations

For biocompatible modules use bio / biocompatible parts only!

Do not mix with bio-inert parts.

Bio LC Systems offer two general valve configurations that decide which of the 2D-LC modes that can be used with the instrument. While the Single Heart-Cutting (SHC) configuration offers access to Single Heart-Cutting and Comprehensive 2D-LC, the Multiple Heart-Cutting (MHC) configurations also give access to Multiple Heart-Cutting and High-Resolution Sampling 2D-LC. The Active Solvent Modulation valve is available for the SHC and MHC configuration. An overview of the recommended Bio 2D-LC mode can be found in the hardware configuration (Recommended Bio Stack Setups on page 95).

Stack setups of all other LC modules (reference) remain valid since those setups are independent of the valve configuration.

Table 15: Overview of 2D-LC modes dependent on valve configuration of the Bio 2D-LC system

Valves	SHC Configuration with ASM Valve	MHC Configuration
		
Bio 2D-LC Valve, Active Solvent Modulation (ASM)	✓	✓
Operation Modes	**SHC Configuration with ASM Valve**	**MHC Configuration**
Comprehensive (LCxLC)	✓	✓
Single Heart-Cutting	✓	✓
Multiple Heart-Cutting	X	✓
High-Resolution Sampling	X	✓

Single Heart-Cutting Configuration

BIO

For biocompatible modules use bio / biocompatible parts only!

Do not mix with bio-inert parts.

Biocompatible 2D-LC systems that are exclusively used for Single Heart-Cutting and Comprehensive 2D-LC experiments require the 2D-LC ASM valve. The valve can be conveniently attached to any pump that is installed. For an SHC configuration, transfer capillaries are not necessary since MHC decks are not installed.

Figure 45: Schematics of a Single Heart-Cutting (SHC) Configuration with supported valves

NOTE For the Bio 2D-LC setup (Single Heart-Cutting (SHC) with ASM Valve), LC driver 3.5 (or higher) is required.

NOTE Due to the increased wear, ASM functionality is not recommended for comprehensive runs in SHC or MHC configuration.

Multiple Heart-Cutting Configuration

BIO

For biocompatible modules use bio / biocompatible parts only!

Do not mix with bio-inert parts.

Biocompatible 2D-LC Systems that are used for Multiple Heart-Cutting or High-Resolution Sampling 2D-LC require extra Bio MHC decks. For MHC configurations, the Bio ASM valve head is supported. The valve can be conveniently attached to any bio pump in the stack. For the installation on the valve head, the transfer bio capillaries must be installed as follows.

NOTE The Bio MHC Valve SST (G5642-64000) uses sample loops which have a biocompatible coating on the internal side of the stator and a PEEK rotor for protecting sensitive bio samples.

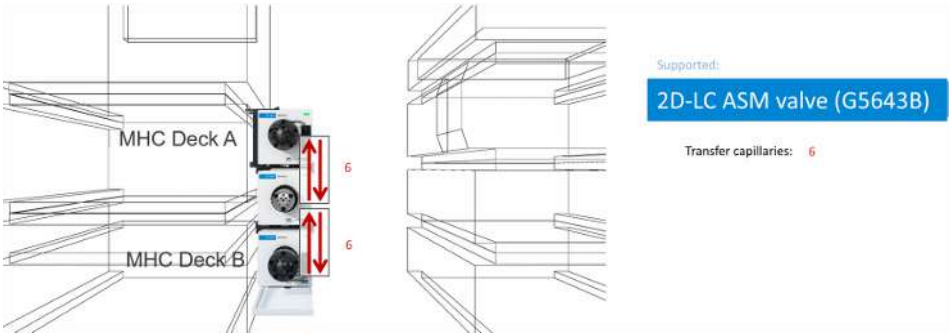


Figure 46: Schematics of a Multiple Heart-Cutting (MHC) Configuration with supported bio valves and bio transfer capillaries

Recommended Bio Stack Setups

Bio 2D-LC Systems allow two basic stack setups. The pumps used for the first and second dimension distinguish the basic stack configurations. In the second dimension, a 1290 Bio High-Speed Pump is mandatory. The pumps are always based on the bottom. Other variations depend on the column compartment concept that is used. The bio capillary kit covers all recommended configurations. The following configurations ensure minimum delay and dispersion volumes and therefore optimize the system flow path:

Table 16: Supported instrument configurations with a list of supported Bio LC pumps. Numbers refer to the recommended bio stack setup

#	^{1}D pump	Supported ^{2}D pumps
1	**1290 Infinity II/III / 1260 Infinity II/III Prime LC** 1260 Bio Flexible Pump (G7131C) 1290 Bio Flexible Pump (G7131A) 1290 Bio High-Speed Pump (G7132A)	**1290 Infinity II/III** 1290 Bio High-Speed Pump (G7132A)
2	**1260 Infinity II/III** 1260 Bio-Inert Quat Pump (G5654A)	**1290 Infinity II/III** 1290 Bio High-Speed Pump (G7132A)

NOTE

This guide only covers setups with bio pumps of the 1290 Infinity II/III series. Setups with other bio modules of the 1200 Infinity Series can require extra bio capillaries.

Connections mentioned in this setup are the following:

- Concurrent direction for the Bio 2D-LC ASM Valve with Single Heart Cut Configuration, see Figure 47 on page 97.
- Countercurrent for the Bio 2D-LC ASM Valve with a Multiple Heart-Cutting Configuration, see Figure 47 on page 97.

⇨ If you want to connect the Bio 2D-LC Valve in another direction than in these recommended 2D-LC setups, please follow the schematics shown under **2D-LC Valve Topologies** in the LC Driver Online help.

Connecting the Bio 2D-LC ASM Valve without MHC

BIO

For biocompatible modules use bio / biocompatible parts only!

Do not mix with bio-inert parts.

The capillary connections of the 2D-LC valves depend on whether a con- or countercurrent configuration is used. For the Bio ASM Valve, both concurrent and countercurrent operation are possible. Schematics in this chapter will reflect a concurrent direction.

If you want to connect the Bio ASM Valve in a different direction, follow the schematics shown under **2D-LC Valve Topologies** in the LC Driver Online help.

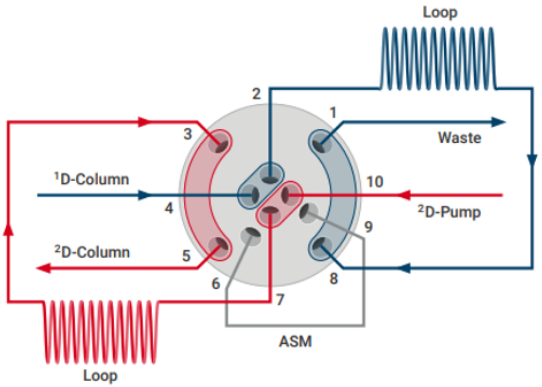


Figure 47: Schematic representation of the Bio 2D-LC ASM Valve without MHC in concurrent flow

NOTE For the ASM functionality of the Single Loop Set up, the installation of transfer capillaries is recommended.

NOTE Bio 2D-LC ASM Valve without MHC requires LC drivers 3.5 (or higher).

Port	Number of Capillary	Connection	ID x L [mm]	P/N	Description
1		Waste line	0.7 x self-cut	0890-1713	Tubing-flexible 0.8/1.61 mm PTFE WT (delivered with UV detector)
2		Sample Loop (blue) (IN)	0.35 x 831	5004-0028	Capillary MP35N 0.35x831 M/M 80 µl see port 8 (This is an example and can be replaced by any other sample loop)
3		Sample Loop (red) (OUT)	0.35 x 831	5004-0028	Capillary MP35N 0.35x831 M/M 80 µl see port 7 (This is an example and can be replaced by any other sample loop)
4		from pressure release kit; from ^{1}D column,^{1}D detector	0.12 x 170	5500-1603	Quick Turn Capillary MP35N 0.17x400 M/M
5		to ^{2}D column (Heat exchanger)	0.12 x 170	5500-1597	Quick Turn Capillary MP35N 0.12x400 M/M
6		ASM Capillary e.g. ASM f-3	0.12 x 170	5004-0022	Capillary MP35N 0.12x170 M/M See port 9
7		Sample Loop (red) (IN)	0.35 x 831	5004-0028	Capillary MP35N 0.35x831 M/M 80 µl see port 3 (This is an example and can be replaced by any other sample loop)

Port	Number of Capillary	Connection	ID x L [mm]	P/N	Description
8		Sample Loop (blue) (OUT)	0.35 x 831	5004-0028	Capillary MP35N 0.35x831 M/M 80 µl see port 2 (This is an example and can be replaced by any other sample loop)
9		ASM Capillary e.g. ASM f-3	0.12 x 170	5004-0022	Capillary MP35N 0.12x170 M/M See port 6
10		from ^{2}D pump	0.17 x 400	5500-1603	Quick Turn Capillary MP35N 0.17x400

Connecting the Bio 2D-LC Valve, ASM with MHC

For biocompatible modules use bio / biocompatible parts only!

Do not mix with bio-inert parts.

In contrast to the Bio 2D-LC ASM Valve in SHC configuration Agilent recommends using a counter-current setup for the Bio 2D-LC ASM Valve in MHC configuration. This section describes the setup for a counter-current configuration of the Bio 2D-LC ASM Valve. For the concurrent setup, please refer to concurrent configuration of the ASM 2D-LC Valve in the 2D-LC Software. You find the Valve topology configuration screen in OpenLab ChemStation under Instrument > 2D-LC Configuration or in OpenLab CDS and MassHunter under 2D-LC Valve Topologies in the LC Driver Online help.

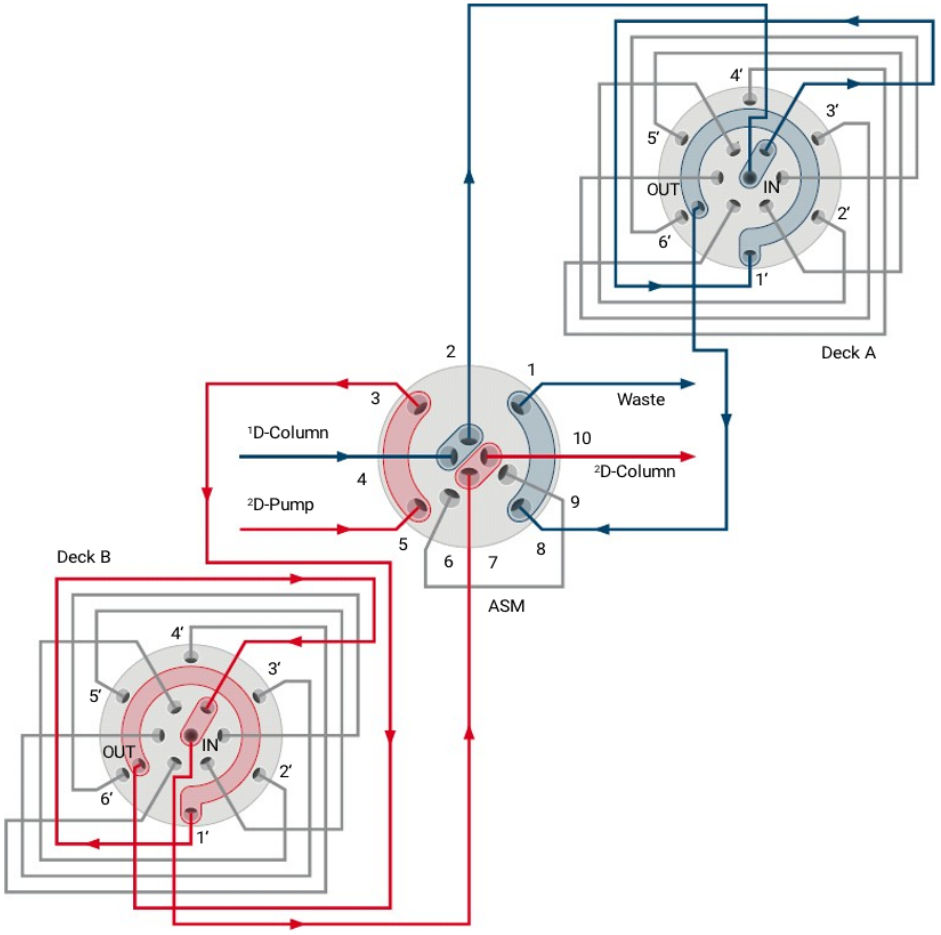


Figure 48: Schematic representation of the Bio 2D-LC ASM Valve in countercurrent flow

NOTE Against the example shown in the figure above, for 1200 bar MHC Valves that have a different symmetry, the connection is OUT/IN.

Port	Number of Capillary	Connection	ID x L [mm]	P/N	Description
1	11	waste line	0.7 x self-cut	0890-1713	Tubing-flexible 0.8/1.61 mm PTFE WT (delivered with UV detector)
2	6	Bio transfer capillary to MHC Valve (IN), deck A	0.12 x 170	5004-0020	Capillary MP35N 0.12x170 M/M

Port	Number of Capillary	Connection	ID x L [mm]	P/N	Description
3	6	Bio transfer capillary from MHC Valve (OUT), deck B	0.12 x 170	5004-0020	Capillary MP35N 0.12x170 M/M
4	5 F3	from pressure release kit; from ^{1}D column, ^{1}D detector	0.17 x 400	5500-1603	Quick Turn Capillary MP35N 0.17x400 M/M
5	9	from ^{2}D pump	017 x 400	5500-1603	Quick Turn Capillary MP35N 0.17x400 M/M
6	ASM1-4	outlet to Bio ASM capillary	0.12 x L		see list below
7	6	Bio transfer capillary to MHC Valve (IN), deck B	0.12 x 170	5004-0020	Capillary MP35N 0.12x170 M/M
8	6	Bio transfer capillary from MHC Valve (OUT), deck A	0.12 x 170	5004-0020	Capillary MP35N 0.12x170 M/M
9	ASM1-4	inlet from Bio ASM capillary	0.12 x L		see list below
10	7	to ^{2}D column	0.12 x 400	5500-1597	Quick Turn Capillary MP35N 0.12x400 M/M

Which Bio ASM capillary (MP35N) shall be used depends on the ASM factor, which is optimum for your application. You may choose from following capillaries:

Table 17: Available ASM Capillaries and properties

Bio Capillary p/n	Length (mm)	Inner diameter (mm)	Volume (µL)	ASM factor	Split ratio (loop:ASM)
5004-0021	85	0.12	0.96	5	1:4
5004-0022	170	0.12	1.9	3	1:2
5004-0023	340	0.12	3.8	2	1:1
5004-0024	680	0.12	7.7	1.5	1:0.5

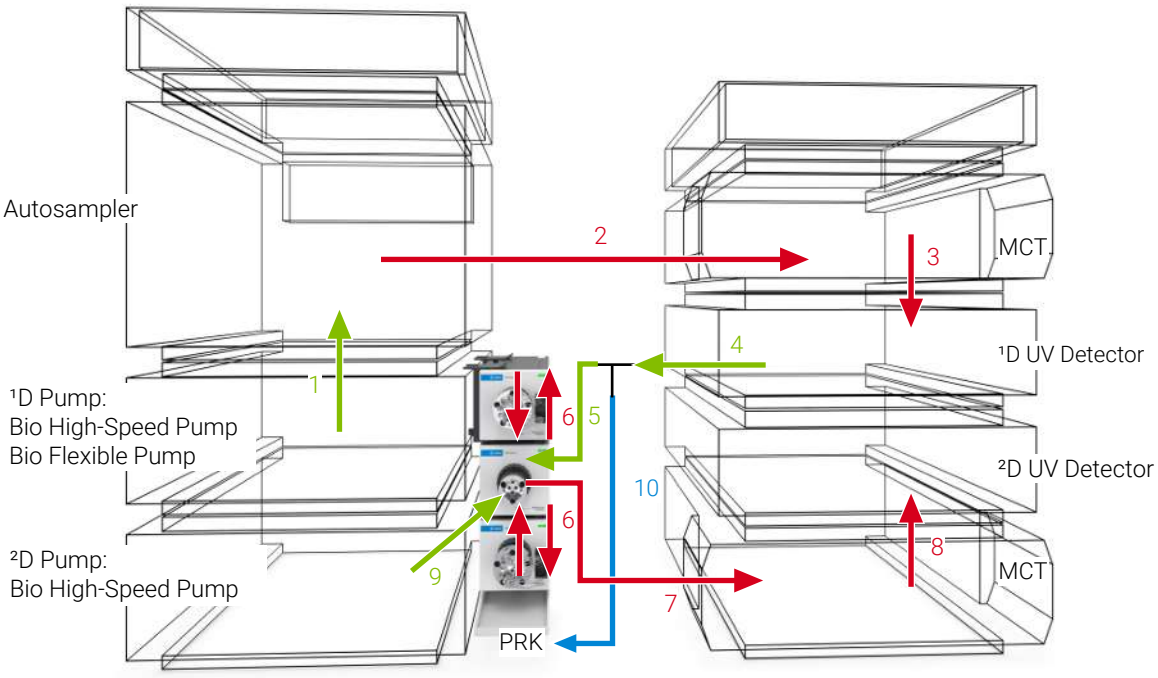


Figure 49: Recommended setup if both bio pumps are Infinity II/III modules or the ²D pump is a 1290 Bio High-Speed Pump

Number of Capillary	#	Connection	ID x L [mm]	P/N	Description
1	1	¹D pump (top) to autosampler	0.17 x 400	5500-1603	Quick Turn Capillary MP35N 0.17 x 400 M/M
2	1	Autosampler to Bio Quick-Connect Heat Exchanger Standard Flow (MCT1)	0.12 x 600	5004-0031	Capillary MP35N 0.12 x 600
	1	Bio Quick-Connect Heat Exchanger Standard Flow to ¹D column (in MCT1)	0.12 x 105	5500-1578	Quick-Connect Capillary MP35N 0.12x105 M/M
3	1	¹D column to ¹D detector	0.12 x 400	5500-1597	Quick Turn Capillary MP35N 0.12 x 400 M/M
4	1	¹D detector to T-piece of PRK	0.17 x 105	5500-1599	Quick Turn Capillary MP35N 0.17 x 105 M/M

Number of Capillary	#	Connection	ID x L [mm]	P/N	Description
5	1	T-piece of PRK to Bio 2D-LC ASM Valve (Port 4)	0.17 x 400	5500-1603	Quick Turn Capillary MP35N 0.17 x 400 M/M
6	4	Bio 2D-LC ASM Valve (Port 7) - Deck (IN), Deck (Out) - Bio 2D-LC ASM Valve (Port 3) Bio 2D-LC ASM Valve (Port 2) - Deck (IN), Deck (Out) - Bio 2D-LC ASM Valve (Port 8)	0.12 x 170	5500-1376	Capillary ST 0.12 x 170 M/M (delivered with 2D-LC Valve Kit, ASM)
7	1	Bio 2D-LC ASM valve (Port 10) to Bio Quick-Connect Heat Exchanger Standard Flow (MCT1 or 2)	0.12 x 400	5500-1597	Quick Turn Capillary MP35N 0.12 x 400 M/M
	1	Bio Quick-Connect Heat Exchanger Standard Flow to 2D column (in MCT1 or 2)	0.12 x 105	5500-1578	Quick-Connect Capillary MP35N 0.12 x 105 M/M
8	1	^{2}D column (in MCT 1or 2) to ^{2}D detector	0.12 x 280	5500-1596	Quick Turn Capillary MP35N 0.12 x 280 M/M
9	1	^{2}D pump to Bio 2D-LC ASM Valve (Port 5)	0.17 x 400	5500-1603	Quick Turn Capillary MP35N 0.17 x 400 M/M
10	1	T-connector of PRK to damper capillary	0.17 x 150	5500-1600	Quick Turn Capillary MP35N 0.17 x 150
	1	Bio 2D-LC ASM Valve (Port 1) to Waste (not shown)	0.7 x self-cut	0890-1713	Tubing-flexible 0.8/1.61 mm

NOTE InfinityLab Quick Turn fittings require the capillaries specified in this table.

Alternative Instrument Setups for Additional Functionality

For biocompatible modules use bio / biocompatible parts only!

Do not mix with bio-inert parts.

The driver-based Bio 2D-LC Solution allows only certain valves to be configured as bio diverter valves which can be used for example as an effective desalting tool.

More information is available in the following sections:

- Method Parameters on page 152
- Run the System on page 228

Table 18: Supported valves

Description	P/N
2-position/6-port valve head, 600 bar, bio-inert	5067-4148
2-position/10-port valve, bio 1300 bar, PEEK, MP35N	5067-6682

D

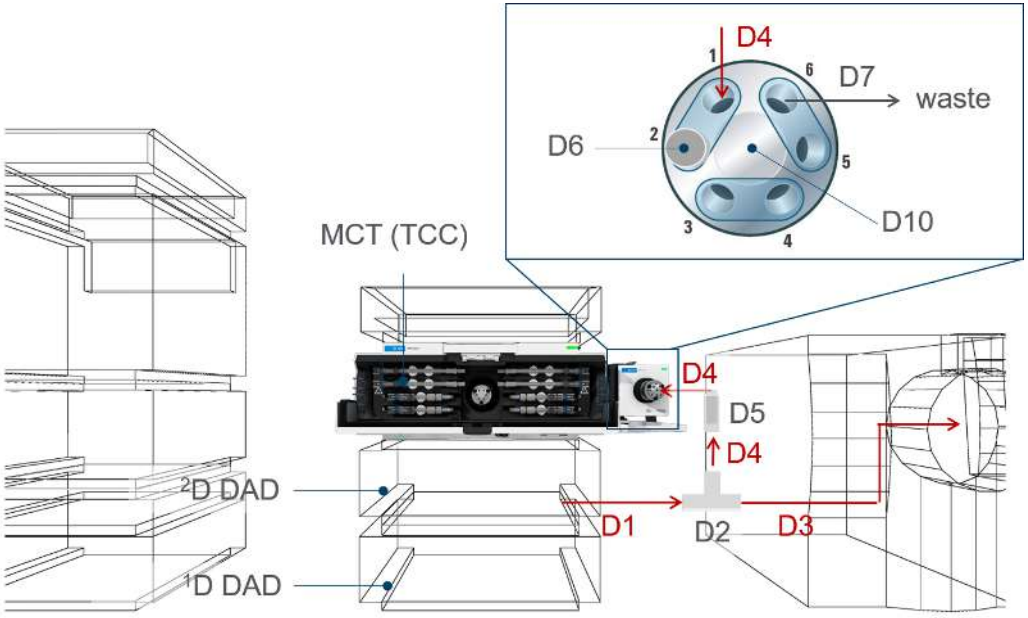


Figure 50: Recommended setup of a MS diverter valve

Table 19: Available capillaries

Number of Capillary	#	Connection	ID x L [mm]	P/N	Description
D1	1	Capillary from 2D detector to T-piece	0.12 x 400	5500-1597	Quick Turn Capillary MP35N 0.12x400
D2	1	T-piece (PEEK includes fittings)		5022-2144	1/16in Tee, SST, Low Dead Volume
D3	1	Capillary from MS to T-piece (self cut)	0.12 x 400	0890-1915	Capillary PEEK, 0.12x1250
D4	2	T-piece to pressure relief valve; pressure relief valve to diverter valve	0.3 x 80	5500-1473	Capillary MP35N 0.3x80 SL/SL
D5	1	Pressure relief valve		G4212-60022	Pressure relief valve
D6	1	Blank nut		5043-0277	Blanking Nut long 10-32
D7	1	Diverter valve to waste (Waste line)		0890-1713	Tubing-flexible 0.8/1.61mm PTFE WT
D8	1	Peak fittings		5063-6591	Fitting-Fingertight PEEK for 1/16-in
D9	1	Valve holder for Valve drive to attach to MCT		5067-6138	Valve Holder Kit Right-IF-II-G
D10	1	Diverter Valve		G5631A	2-position/6-port valve head, 600 bar, bio-inert
				G5641A	2-position/10-port valve, bio 1300 bar PEEK, MP35N

For all other capillaries / connections, see:

- Figure 33 on page 68,
- Figure 34 on page 69 , and
- Figure 35 on page 70.

NOTE To be recognized as a diverter valve in the driver-based 2D-LC solution, the diverter valve must be installed in an external valve drive (G1170A).

Installing the Pressure Release Kit

BIO

For biocompatible modules use bio / biocompatible parts only!

Do not mix with bio-inert parts.

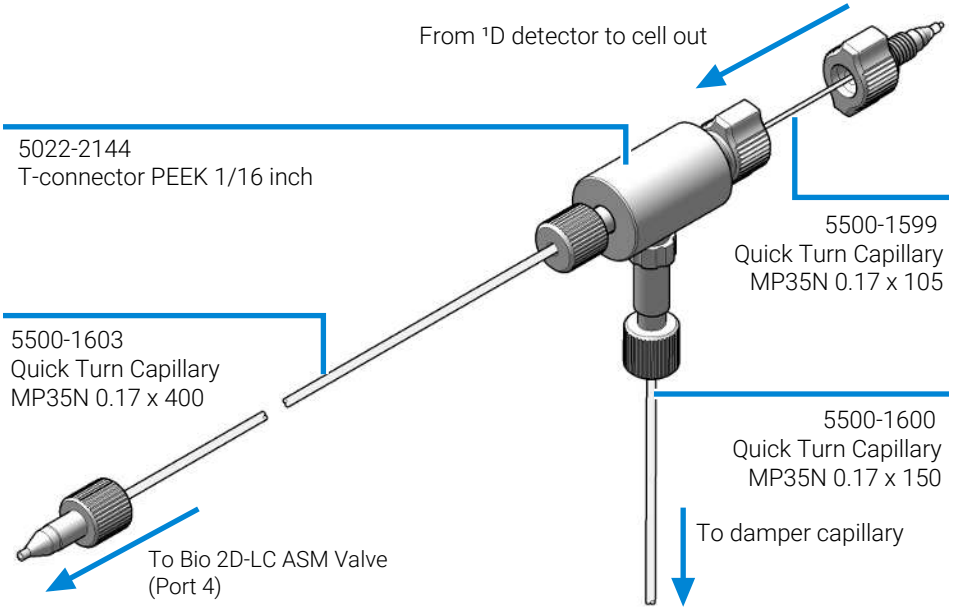


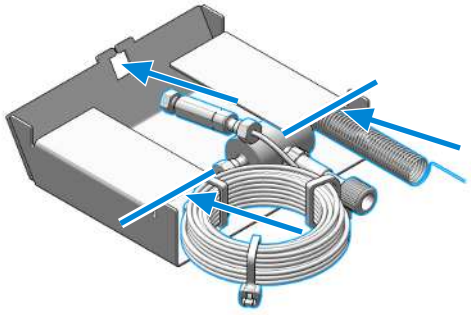
Figure 51: Connections to the pressure release kit

Parts required	Qty.	p/n	Description
	1	G4236-60010	2D-LC Pressure Release Kit

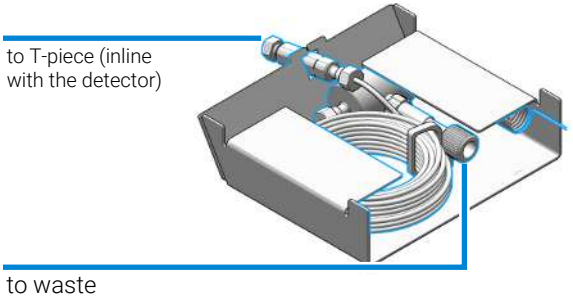
NOTE For the bio 2D-LC system, a T-connector PEEK is included which should then be exchanged for full bio compatibility.

NOTE With the use of the T-connector PEEK in the flow path, there is a pressure limit of 600 bar at this point.

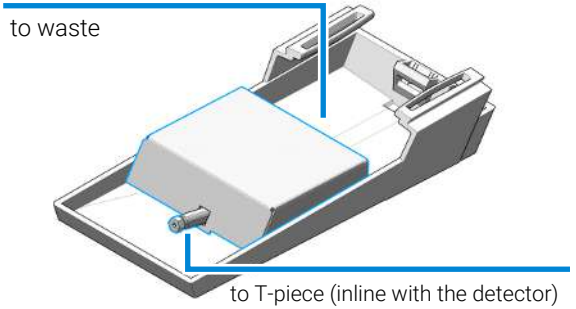
1 Push the pressure release valve assembly in the frame.



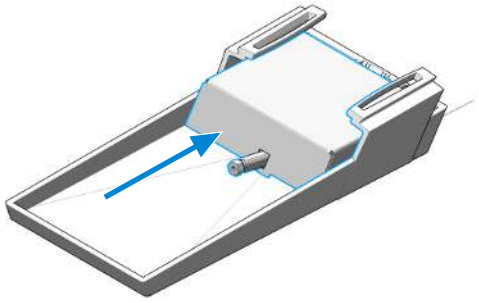
2 Take care for the correct orientation.



3 Insert the pressure release assembly to the leak tray, orientation as shown.



4 Push the pressure release assembly in the correct position.



5 Connect with the T-piece, see Figure 51 on page 105.

Install the Valve Head and Connecting Capillaries

For instructions on how to install the valve head and connecting capillaries, see the user manual.

NOTE

For alternative instrument setups with extra functionality, please see the 2D-LC User Manual or the standard quick installation guide, which gives an overview.

Recommendations for Biocompatible and Bio-Inert Systems

- Make sure all supplies (fittings, capillaries, inline filters, columns, etc.) are bio-inert or biocompatible.
  - Be aware that even columns recommended for bio applications may have a stainless steel case and can introduce iron and other metal ions in the flow path. This material in the flow path may lead to adsorption of susceptible samples like phosphorylated nucleotides. In this case, use PEEK-lined columns.
- After using the system with solvents or samples containing salts, flush it extensively with water to prevent blockages caused by salt crystals.
- If pressure falls below 20 bar, reliable operation of 1290 pumps during analysis cannot be guaranteed. For optimal results, pressure should be at least 50 bar continuously. Therefore, when using columns that create low

backpressure (< 50 bar, such as SEC columns with 1290 LC systems), install a restriction capillary between the pump and the sampler, to achieve at least 50 bar.

- Perform daily flush of the Multisampler with water if the Multiwash Option is installed (see *Best Practices for Using an Agilent LC System Technical Note (InfinityLab-BestPractice-en-SD-29000194.pdf, SD-29000194)*).

CAUTION

Agilent Bio-inert and Bio LC systems should not be subject to passivation or similar procedures

This can cause irreversible damage to the system's internal surfaces

— Do not perform passivation or similar procedures on bio-inert and biocompatible systems.

Flushing Procedure

- Perform this procedure regularly, when salt-containing mobile phases are used. To remove salt deposits from the flow path and surfaces in contact with the solvents, repeat the procedure regularly. Repeat the procedure at least once a week, or prior a long standby or off time. How to prepare the system for shutting down, see section *Shut Down the System* in the Bio LC user manual.
- The procedure is mandatory for switching from salt-containing mobile phase to reversed phase applications (or any applications running with high organics), where the precipitation of salt can occur.
- Flush the column with recommended storage solvent, be sure that this solvent is compatible with current mobile phase and cannot cause precipitation.
- Replace the column with a union, replace the salt-containing solvent bottle with a new bottle of HPLC-grade water at room temperature.
- Clean the bottle head assembly using lint-free wipes to minimize carry over of remaining salt solution into the new water bottle.
- Autosampler: Perform at least 15 min purge with water. This measure removes salt residues from all lines, both needle wash and seat backflush for Multiwash Option. Visually control needle/seat/washport for salt residues, if necessary manually clean needle/seat/washport.
- Purge each pump channel that has pumped buffer separately, for at least 10 min at 5 mL/min.

- Flush the entire system flow path with water for at least 10 min at 2 mL/min. During this step, switch the injection valve and the column selection valve (if installed) position every 1 min. Repeat this step until every position has been selected for at least five times.
- To minimize salt carry over, replace water with fresh solvent bottles.

Licensing the 2D-LC Instrument in MassHunter

To use the driver-based 2D-LC solution, you need the following licenses:

- MassHunter License, and
- 2D-LC USB hardware dongle

Activate the 2D-LC System Driver With a License Dongle

When you purchase Agilent driver-based 2D-LC Software from Agilent you will receive a single USB stick which includes the 2D-LC dongle license. To run the system and use its functionality, the ^{2}D pump must be activated. For this purpose, the physical device is connected to the USB-port on the back of the 2D-LC pump. This will activate and enable the 2D-LC acquisition feature in the LC driver and allow the Agilent 2D-LC Software to be used in your CDS.

Parts required

Qty.	p/n	Description
1		USB Dongle This Dongle is a software license of significant value. Agilent will not replace lost or damaged dongles. Store it in a safe place. Write down the serial number of the module activated with this dongle.

The ^{2}D pump must be a 1290 Binary Pump, or any 1290 Infinity II/III High-Speed Pump.

Activate the Agilent 2D-LC Acquisition Feature in the LC Driver

1. Power off the module.
2. Plug the USB Dongle into the ^{2}D pump on the back of the module.
3. Power on the module.
4. Once restarted, the 2D-LC License is activated and you can remove the USB Dongle and store in a safe place.

NOTE The dongle is required for a re-activation of 2D-LC License after mainboard replacement.

NOTE When the 2D-LC Driver connects to the instrument, it checks if a license is available. If no licence is available, the driver remains offline. Tooltip when hovering over the 2D-LC UI in the dashboard of the CDS shows the text: No 2D-LC license available.

For further information, see Agilent Lab Advisor Software on page 310.

Deactivate the License (Deactivation Steps in LabAdvisor)

For the deactivation of the license on the 2D-LC pump (e.g. you want to use the license on a different 2D-LC pump) you have to use the LabAdvisor Diagnostic Software.

1 Insert the USB Dongle at the rear of the ^{2}D pump.

2 Deactivate the license under Instrument Control > Pump > Special Commands > License Dongles .

3 Remove the USB dongle at the rear of the pump and keep it on a safe place.

For further information, see Agilent Lab Advisor Software on page 310.

2D-LC Software Installation and Configuration in Agilent Masshunter Workstation

Preparations

- A compatible CDS must be installed first. For details, see the respective CDS documentation.

  To observe if your computer fulfills the requirements, e.g. the hardware CPU, memory, hard disk space, and the software, check the windows settings.

  Check that your Windows operating system supports 2D-LC solution. For details, see section Supported Operating Systems on page 46.

  It is recommended to use the *Agilent MassHunter Workstation Requirements Guide (MHRequirements_EN.pdf, D0026036)* and the *Agilent 1290 Infinity II 2D-LC System MassHunter User Guide (G2198-2D-LC-MassHunter-UseMa-en-D0028445.pdf, D0028445)* as guidance for the installation.
- Set up the computer system
- Check PC network card configuration
- Prepare for installation
- To make sure you have the latest critical updates and security fixes, run Windows Update
- Make sure that Windows Update is completed before you continue

NOTE

- If you are upgrading from MassHunter 10.x build, uninstall MassHunter 10.x first.
- Be sure that there has been no other MassHunter installations on the PC or you will need to re-image.
- Be sure *ALL Windows Updates* are COMPLETED before Installing a build the first time or you may have to re-image again.
- Decide on the installation type. For 2D-LC, use the noncompliant workstation. *This decision is permanent and can only be changed by re-image*.

NOTE

Combination of MassHunter Workstation higher than version 10 and ChemStation with 2D-LC add-on software on one PC is not recommended.

NOTE For 2D-LC setups only the MassHunter workstation will work. Network Workstation does not support 2D-LC because it does not support 2D-LC File Splitter Automation.

1 Install the Data Acquisition program.

2 Install the Qualitative Analysis program.

3 Install the Quantitative Analysis program.

4 **Optional:** Install Microsoft Excel.

5 Install Service Packs for Data Acquisition.

6 Install Quantitative Analysis Reporting.

7 **Optional:** Configure Excel for MassHunter.

NOTE This configuration is mandatory to avoid any issues later. Usually, the CDS installs a driver, which however may not be the latest one and may require a driver update in the next step.

8 To update the LC & CE Drivers in MassHunter, follow the instructions in the MassHunter installation document.

9 If the CDS has already been installed:

Check, see Compatibility Matrix on page 45, that the following components are compatible with the 2D-LC solution:

- Software
- LC driver
- Firmware

10 Install Lab Advisor Diagnostic Software and Update the firmware for the entire LC system, see Replace the Module Firmware on page 324.

For the minimum required firmware set, see Supported Firmware on page 51.

Additional Information

Installation and User Guides

Use the corresponding Quick Start Guide to familiarize yourself with the LC/MS instrument and for your first steps using the instrument:

- Agilent 6400 Series Triple Quadrupole LC/MS Quick Start Guide
- Agilent 6200 Series TOF and 6500 Series Q-TOF LC/MS Quick Start Guide

The Quick Start Guides also contain a detailed list of documentation that will help you become further acquainted with the MassHunter software.

NOTE

Guides are available in the corresponding resource app:

— TOF and Q-TOF Resources

— TQ LCMS Resources

— LCMS Data Analysis Resources

⇨ A complete list is available at www.agilent.com.

Training

Use the material in the resource apps to learn to use your MassHunter Workstation software, and to learn, maintain, and troubleshoot your LC/MS instrument.

Visit www.agilent.com to view a list of training courses for your LC/MS instrument.

Best Practice for Using an LC System

The technical note *Best Practices for Using an Agilent LC System Technical Note (InfinityLab-BestPractice-en-SD-29000194.pdf, SD-29000194)* describes best practices like daily and weekly tasks for using an Agilent LC.

Online Help

- To get more information about a window or dialog box, place the cursor on the window or dialog box of interest and press **F1**.

- In the Agilent MassHunter IM-MS Browser program, you instead click **Help** > **Contents** .

  From the **Help** menu, access **How-to** help and reference help.

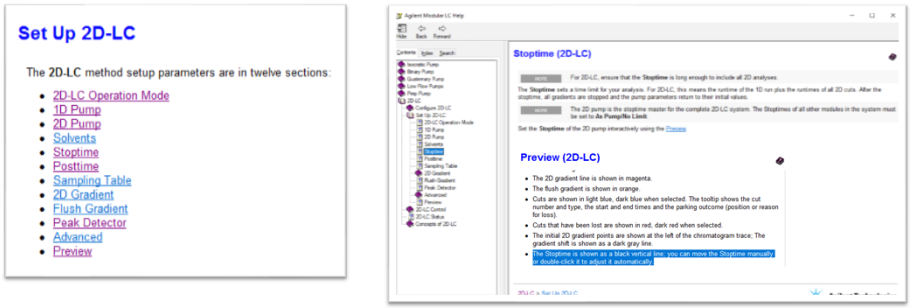


Figure 52: Modular LC help for 2D-LC

Start the Configuration Dialog

Preparations

- The 2D-LC hardware is correctly set up and the system configuration, the project settings and the most instrument settings like the IP Addresses are already defined.

1 Open the Control Panel.

2 Double-click the **Configure Instrument** tool.

OR: Right-click and select **Configure Instrument** .

Figure 53: Configure Instrument view of the Control Panel

3 Select Module/Module Package if not already defined and add **Agilent 1100/1200/1260/1290 LC** as Agilent System.

The following default IP addresses appear in the connection info:

- 192.168.254.12 for the High-End mass spectrometer, and
- 192.168.254.11 for the LC instrument

4 To configure the instrument, use the Instrument Configuration dialog:

a **Optional:** To change the name of the instrument, type a new Instrument name.

b To configure the LC instrument, click Device Config....

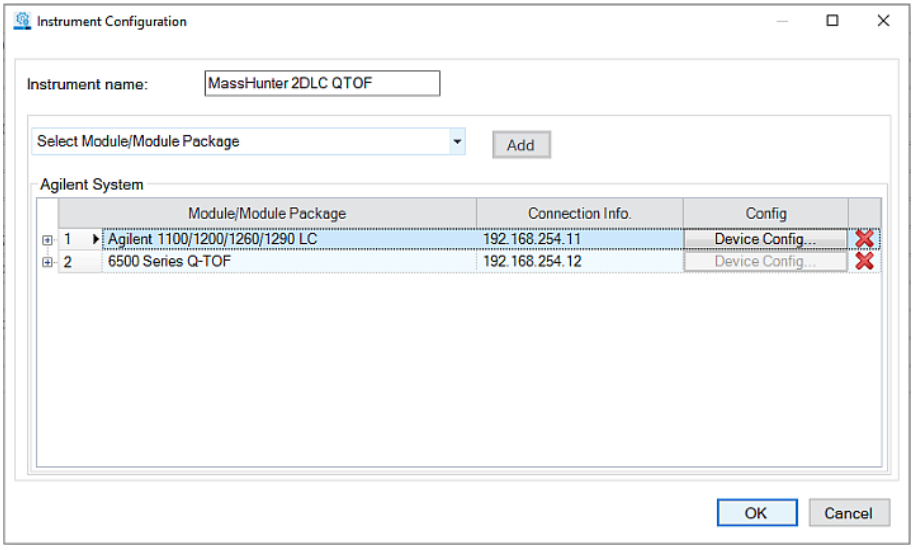


Figure 54: MassHunter Instrument Configuration window using Q-TOF as an example

The Auto Configuration dialog opens.

Configure the HPLC Instrument

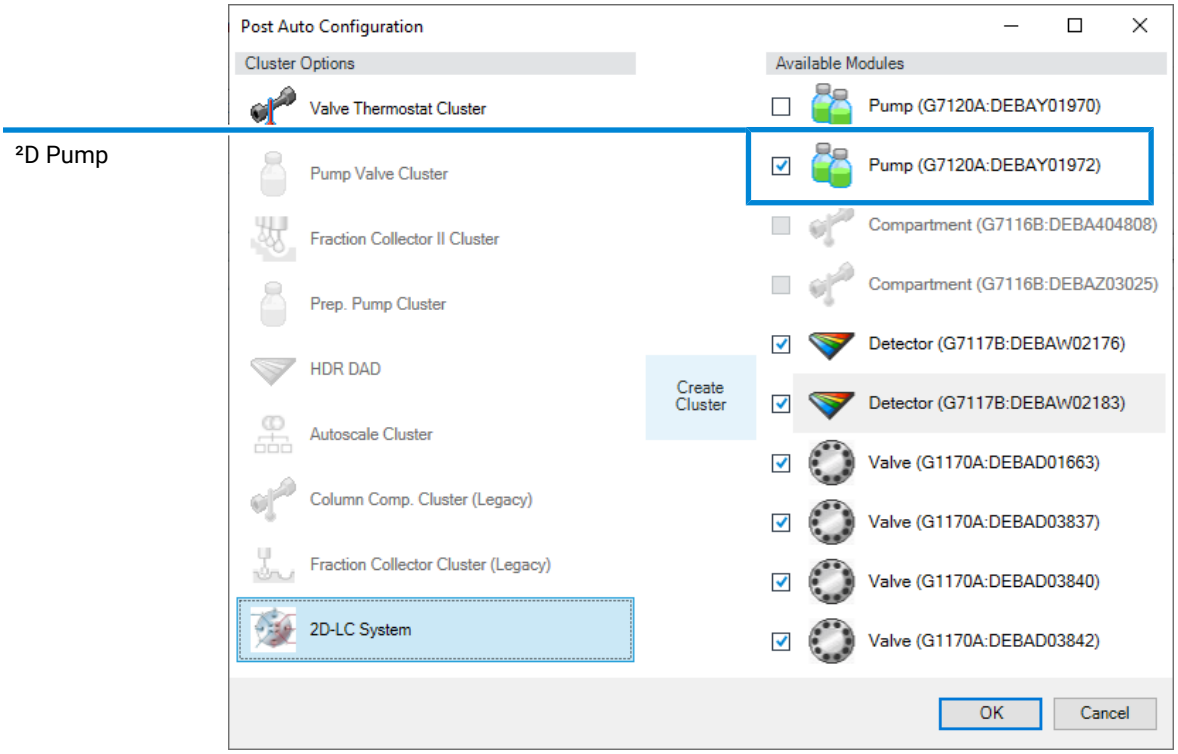


Figure 55: Auto Configuration window of a full 2D-LC solution with two MHC valves and a diverter valve

1 Check/Select **2D-LC System** in **Cluster Options**.

2 Uncheck the ^{1}D pump in **Available Modules** if two binary pumps (for example G4220A/B, G7120A, or G7132A) are installed.

3 To create a cluster, click the **Create Cluster** button.

The **2D-LC Cluster Configuration** window opens.

Configure the 2D-LC Cluster

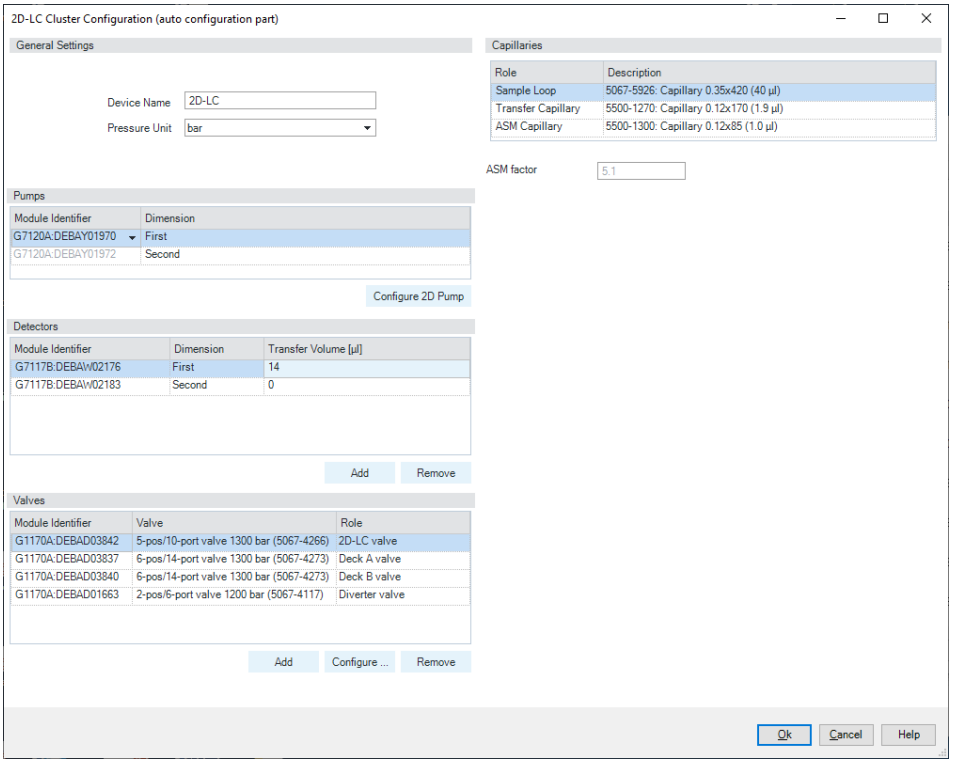


Figure 56: 2D-LC Cluster Configuration (for an ASM Valve, MHC Valves and a Diverter Valve)

The 2D-LC software configuration window allows the following:

- Verification of the ^{1}D and ^{2}D pump configuration
- Configure ^{2}D pump
- Add and select ^{1}D and ^{2}D detectors and define the transfer volume
- Configure the different valves like 2D-LC Valve Head, MHC decks (if multiple valve heads are available), and diverter valve
- Capillary connections like Sample Loops, transfer, and ASM capillary
- Define ASM factor (if ASM valve is available)

NOTE The 2D-LC Cluster Configuration window can look different depending on what kind of device setup has been installed. For example, for a single sample loop setup an extra check box appears.

1 **Optional:** To change the Device Name, connection settings and the Pressure Units, fill in the according fields.

2 To verify the correct ^{1}D and ^{2}D pump configuration, check the **Pumps** settings.

NOTE If different pumps are available, they can be selected as ^{1}D pump via a drop-down menu.

NOTE This action will not rename your pumps. Enter a descriptive naming during initial instrument setup in the instrument configuration.
For more information, see Configure the HPLC Instrument.
The **Configure 2D Pump** button allows the configuration of the ^{2}D pump like, for example, the solvent types.

3 Select ^{1}D and ^{2}D detector under Detectors.

NOTE This action will not rename your detectors. Enter a descriptive naming during initial instrument setup in the instrument configuration, see Configure the HPLC Instrument on page 118.

If necessary, it is possible to configure and select more than two detectors, for example, a UV detector and an ELSD detector.

The ^{1}D settings for the transfer volumes that determine the time between the ^{1}D detection of the peak and the switching of the 2D-LC Valve, depends on the hardware setup. For a standard 2D-LC with two DADs, the transfer volume is approx. 14 µL.

To calculate the volume, add half the volume of the detector flow cell plus the volume between the detector flow cell and the 2D-LC Valve.

NOTE There are the following options to verify the transfer volume ([1]D Detector to 2D-LC Valve) experimentally more precisely:

- Run a time-based High-Resolution experiment (multiple cuts) over one of the first sample peaks. The cut with the highest abundances then corresponds to the apex of your peak. If there is a shift of the peak to the front or to the back, the difference in volume can be calculated and the transfer volume adjusted.
- Alternatively disconnect the transfer capillary connected to the 2D-LC Valve and connect it to the inlet of the [1]D detector instead. The detectors are then connected in series and the transfer volume can be calculated via the offset of the peak.

NOTE Up to four CAN capable detectors are supported in each dimension.

NOTE Not all detectors to be configured will automatically appear in the configuration window. If you want to configure more detectors, you have to do it manually using the add-on button. Please conform to the following format: first the module number followed by a colon and then the serial number, for example G1390B:US12345678.
The detector entry format must be correct to avoid issues later.

NOTE The detector table must contain at least one detector. For a 2D-LC system that has only one High-End mass spectrometer, for example, the info G6546A:SN1234567 must be entered manually. Set up a system with no configured [2]D detector is not allowed.

NOTE For the MassHunter workflow with a High-End mass spectrometer as another second detector, the detector is usually not visible here. Define this transfer volume (delay) during the file splitting in the data evaluation, see Automated File Splitting on page 253.

4 Verify the **Valves**. Depending on the 2D-LC Valve installed, the Standard 2D-LC (G4236A), the ASM 2D-LC Valve (G4243A), or the Bio ASM 2D-LC Valve (G5643B) will automatically appear.

   a **Optional:** If your system contains Multiple Heart-Cutting decks, specify which valve head corresponds to Deck A or B.

   b **Optional:** If the system contains a diverter valve, specify the role of the valve here. You can define further diverter valve settings in the method, see Specify the Switch Time of the Diverter Valve on page 172.

5 Verify the **Capillaries**. Select by clicking your installed capillaries. Check for correct loop size and correct length of the transfer capillaries. If an ASM 2D-LC Valve is used, define the ASM capillary that defines your split ratio, see Introduction to Active Solvent Modulation (ASM) on page 35.

- Define the **Sample Loop** e.g. default 40 µL Sample Loop p/n 5067-5926 for MHC or p/n 5067-5425 for SHC
- Define the **Transfer Capillary**, e.g., default Capillary 0.12x170 (1.9 µL) p/n 5500-1270 for standard valve or Capillary 0.12x170 (1.9 µL) p/n 5500-1376 for ASM valve
- Define the **ASM Capillary**, e.g., default Capillary 0.12x170 (1.9 µL) p/n 5500-1301 for ASM valve, ASM factor 3

NOTE The selection of the ASM Capillary determines the ASM factor, see Introduction to Active Solvent Modulation (ASM) on page 35.
Therefore the ASM factor value cannot be modified later in the acquisition method.

NOTE Generic capillaries are allowed but must be configured first in Lab Advisor before they show up here, see 2D-LC Capillaries Configuration Tool on page 313.

6 To finish, leave the 2D-LC Cluster Configuration, get to the next window, click **OK**.

Configure the Device UI

1 Define names of modules (device name).

Possible options are, for example, the following:

- Sampler
- Iso Pump (Make up Pump)
- ^{1}D Bin Pump
- 2D-LC
- ^{1}D MCT,
- ^{2}D MCT,
- ^{1}D DAD,
- ^{2}D DAD,

For an example, see Figure 57 on page 126.

2 It is recommended to change order of column compartments and detectors. Use the arrow.

NOTE The recommended order of the modules should be followed for method compatibility reasons.

A meaningful order is helpful for the overview of the dashboard and signal naming (e.g. the detector further left, in qual analysis, will be named as signal 1, e.g. DAD1).

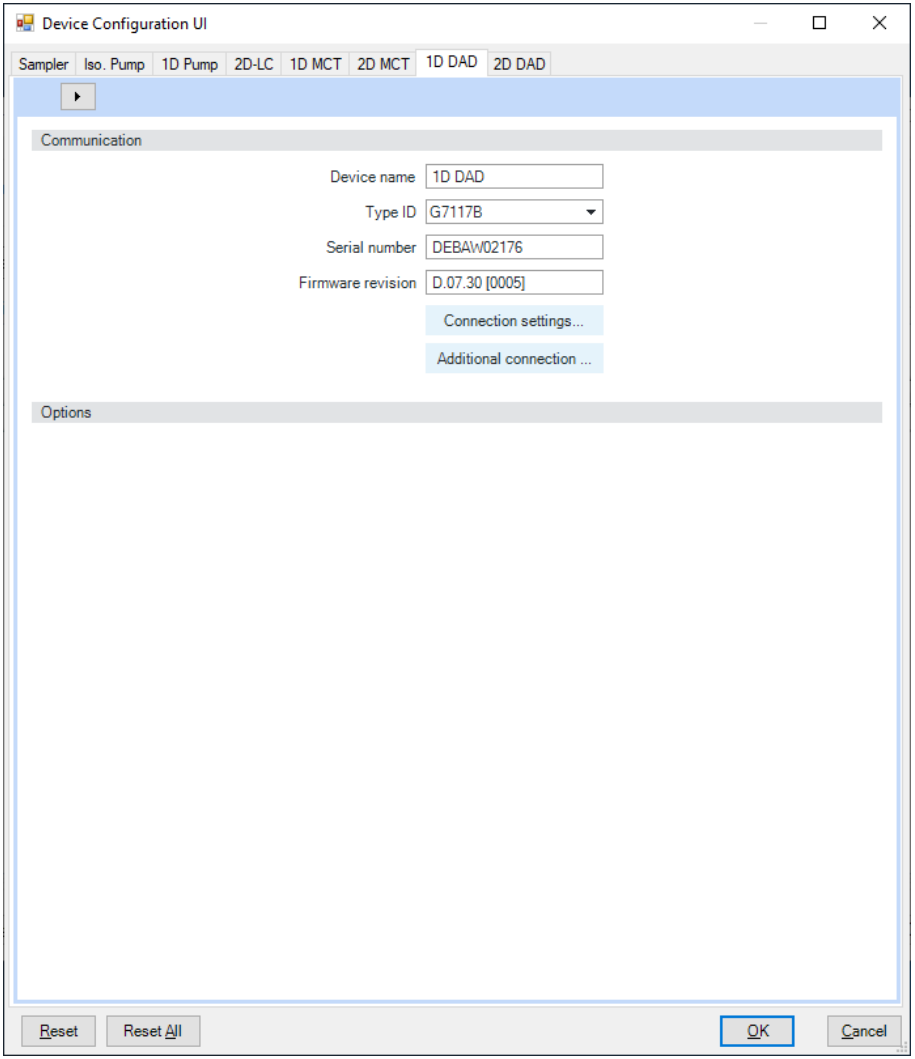


Figure 57: Naming in the Device Configuration for the [1]D detector

Figure 58: Arrangement of the module UI in the dashboard

3 To improve the data rate for each detector, it is recommended to connect both, ^{1}D and ^{2}D, detectors to the LAN. To configure the second detector for the LAN communication, you have to select the detector in the UI and click Additional connection.... Then type in the second LAN address and check Use auxiliary connection.

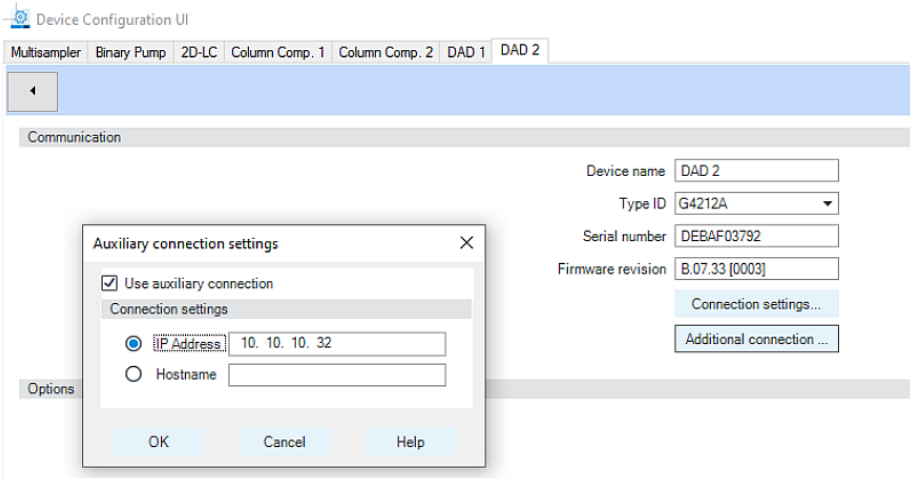


Figure 59: Set up another LAN Connection for the second detector

NOTE For a well-functioning 2D-LC system with two detectors, you need an extra device like a hub or a switch or at least a second LAN card in your PC.

4 When configuration is completed, click **OK**.

5 If instrument is configured successfully, click OK.

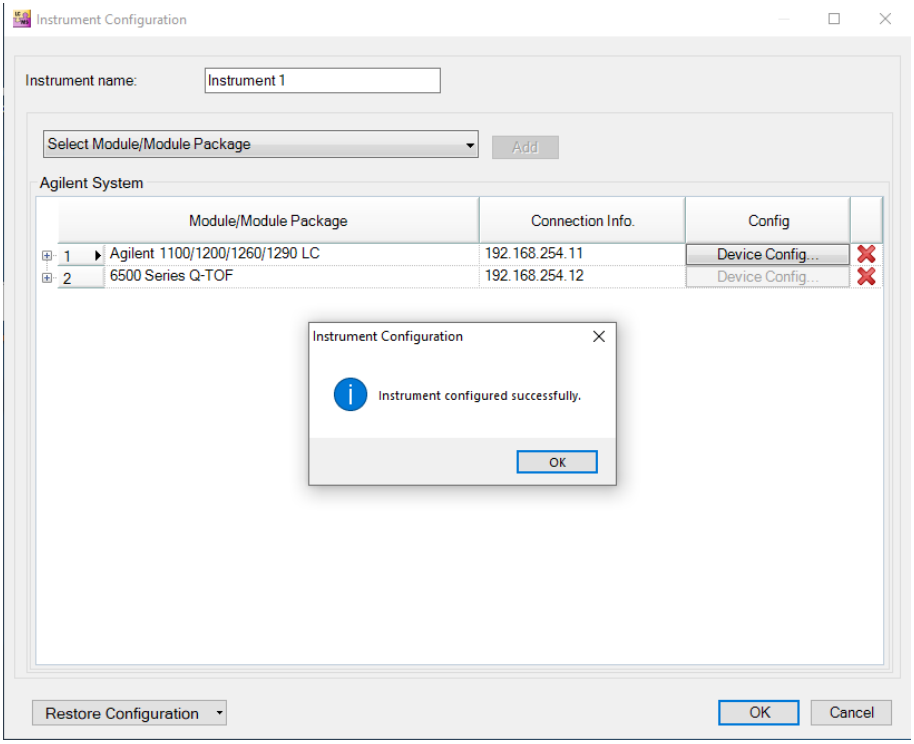


Figure 60: Successful **Instrument Configuration** of a 2D-LC Q-TOF instrument

NOTE If you want to change the 2D-LC cluster configuration later, right click in 2D-LC UI in the dashboard of the CDS.

Important Customer Web Links

- To access Agilent training and education, visit https://www.agilent.com/chem/training to learn about training options, which include online, classroom and onsite delivery. A training specialist can work directly with you to help determine your best options.
- To access the *Agilent Resource Center* web page, visit https://www.agilent.com/en-us/agilentresources. The following information topics are available:
  - Sample Prep and Containment
  - Chemical Standards
  - Analysis
  - Service and Support
  - Application Workflows
- The *Agilent Community* is an excellent place to get answers, collaborate with others about applications and Agilent products, and find in-depth documents and videos relevant to Agilent technologies. Visit https://community.agilent.com/welcome
- Videos about specific preparation requirements for your instrument can be found by searching the *Agilent YouTube* channel at https://www.youtube.com/user/agilent
- *Need to place a service call?* https://www.agilent.com/en/promotions/flexible-repair-options

Further Information

Further information is available:

- Folder **Documents** on the software DVD:
  - Document Primer 2D-LC 5991-2359EN.pdf gives an introduction to principles, practical implementation and applications for Two-Dimensional Liquid Chromatography.
- Folder **Documents** on the Driver CD:
  - Software Status Bulletin (SSB)

  The SSB is updated regularly. Please visit our Websites for the latest version at:

  https://www.agilent.com/cs/library/support/Patches/SSBs/M84xx.html
  - Software Release Bulletin (SRB)

  The SRB is an excerpt from the SSB which lists issues which have been fixed with this revision.
- Firmware and firmware documentation are available for download from https://www.agilent.com/en-us/firmwareDownload?whid=69761.
- Press **F1** in the software user interface for the Online Help with more information on specific software functions.
- For more information about applications, please visite InfinityLab Application Finder https://www.agilent.com/en/promotions/applicationfinder?s=learnmore.
- For more information about Agilent hardware, and software, please visit the Agilent web site at http://www.agilent.com.

5 2D-LC Data Acquisition

This chapter provides information about 2D-LC data acquisition in OpenLab CDS.

2D-LC Data Acquisition in MassHunter Workstation

Start the Data Acquisition Software

Preparations

- To start your instrument, you need the following:
- A configured instrument
- A CDS project associated to the instrument
- Permission to **Run Instrument** included with *Instrument User*, *Instrument Administrator*, or *Everything* role (if authentication is selected)

1 To start the data acquisition, double-click the **MassHunter 2DLC QTOF (online)** icon.

OR: To start the data acquisition, double-click the **Control Panel** icon and click the Launch button in the instrument menu.

When you first start the Data Acquisition software, the main window appears.

Figure 61: Start screen of the MassHunter Workstation software

Since the MassHunter acquisition software uses the same LC driver for different High-End mass spectrometry systems, the windows and UI elements shown here for the 2D-LC Q-TOF setup differ only slightly from those of the 2D-LC TQ.

You do almost all of your work within the different windows of this main window. These windows provide tools to do the following:

- Set up acquisition methods
- Run samples interactively or automatically
- Monitor instrument status and monitor runs

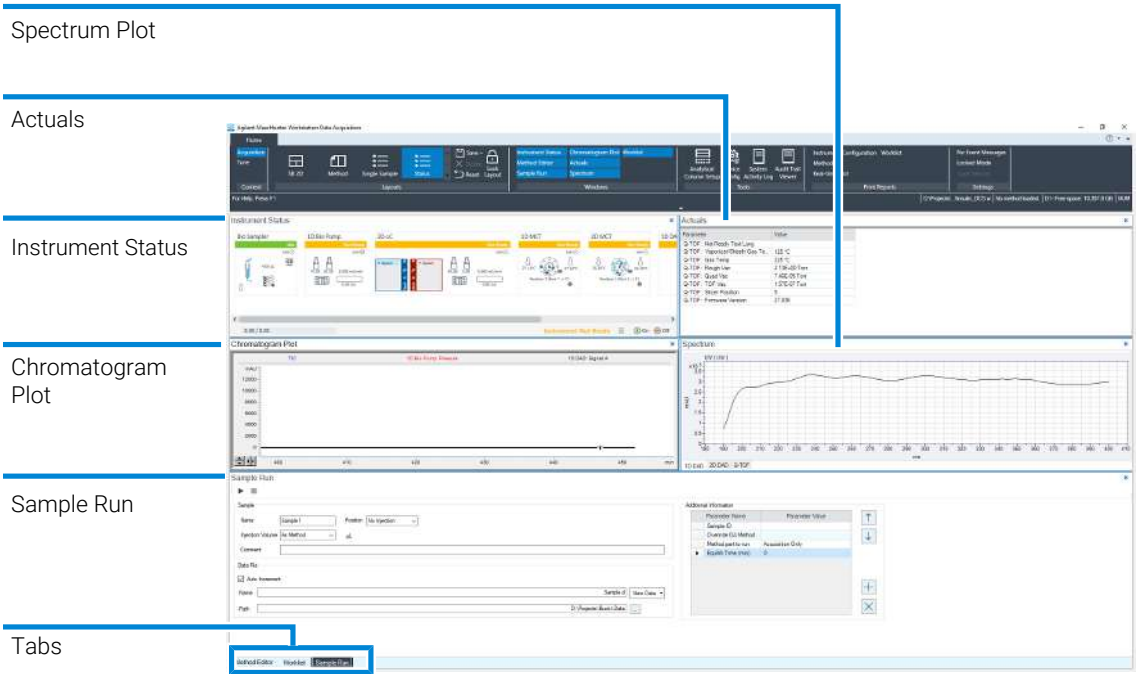


Figure 62: Overview of windows, that are available in MassHunter Workstation Data Acquisition (to switch to different windows, click options under tabs) using the example of a 2D-LC Q-TOF system

NOTE For further help with Q-TOF questions, see *Agilent 6200 Series TOF and 6500 Series Q-TOF LC/MS Quick Start Guide (usermanual-quick start-tof q-tof-G3335-90268EN-agilent.pdf, G3335-90268)*.

Overview 2D-LC in MassHunter Workstation

The dashboard is the common UI element for instrument control.

The driver is responsible for hardware-related features plugged in to the CDS software. These are for example the following:

- Instrument configuration
- Instrument control
- Method parameters
- Instrument status display

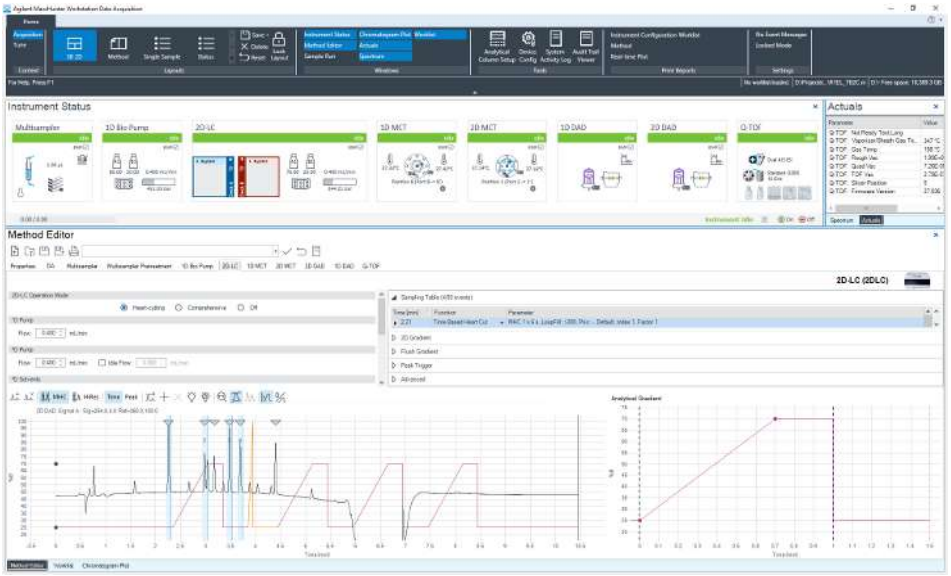


Figure 63: Main Window using the example of a 2D-LC Q-TOF system, the specific 2D-LC UI, and the 2D-LC Method Editor

Instrument Status

The **Instrument Status** window shows the status of each device configured with the instrument. The possible values for Status are shown in the following figure. You also set nonmethod control and configuration parameters for the LC devices and the MS instrument.

Figure 64: Instrument Status for a full 2D-LC Solution

A shortcut menu is available for each device. This window displays each device's status both as text and by its color-coding:

Figure 65: Color code for status

2D-LC User Interface

The instrument status window shows the current state of each of the device. The 2D-LC device is in this example not ready. You can click the button in any device pane to get help on that device. The icons and the information box are visible when you hover over that. In this case, the drive of the binary pump is off. Click the green **On** button in the UI will activate the pump.

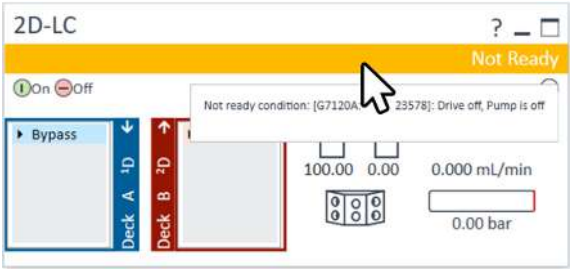


Figure 66: 2D-LC help information

Additional Information in the 2D-LC User Interface

The instrument dashboard can offer some additional settings and information.

In the full view of the 2D-LC UI, a box of actuals is visible. This additional view can be displayed by hovering over the panel and click the square on the upper right side of the interface. Then several instrument signals like flow and pressure etc. show up. By clicking in the corner again, you can undo the step and the box will disappear.

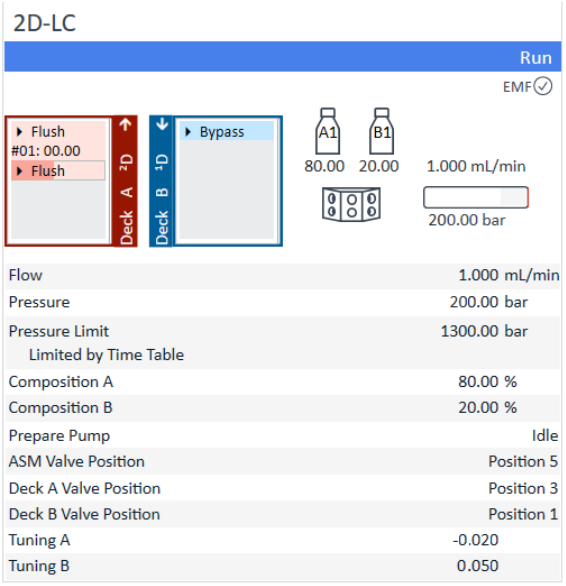


Figure 67: Full view of the 2D-LC UI

Flow	The current solvent flow rate (in mL/min).
Pressure	The current pump pressure (in bar, psi or MPa)
Pressure Limit	The current maximum pressure limit.
Composition A:B	The current solvent composition. When a solvent selection valve is fitted, the channels are shown in the graphic.
Prepare Pump	The info represents the current pump status.
2D-LC Valve position	The info represents the current 2D-LC Valve status. In the current setup an ASM Valve (Position 1-5) is installed see Connecting the 2D-LC Valve, ASM (G4243A) on page 65
Deck A Valve position	The info represents the current MHC Valve status Deck A (Position 1-6)

Deck B Valve position	The info represents the current MHC Valve status Deck B (Position 1-6)
Diverter Valve Position	The info represents the current valve position (Position 1 → Into MSD, Position 2 → Into waste).
Tuning	The signal represents the current effort the pump drives have to take to maintain the current system status.

NOTE For further information, see the pump user manual.

Further information and setting options are available in the Context Menu. For example, you have access to module control and capillaries settings in modify. To make the context menu visible, you have to right click in the UI. In this view, there are several hardware-related features available like the following:

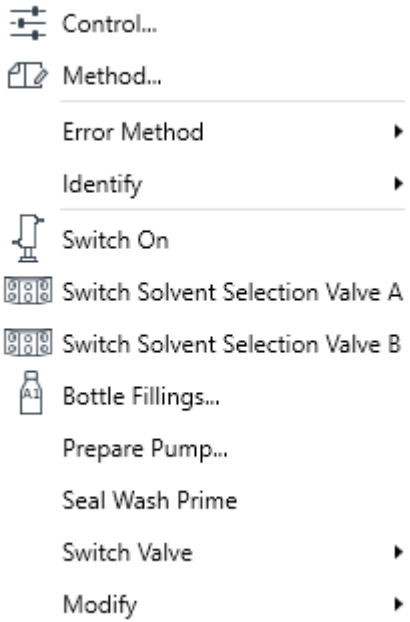


Figure 68: Context Menu / Control Interface of the 2D-LC Cluster

Control	Displays the pump's Control dialog box.
Method	The pump's Method Setup dialog box is only visible in OpenLab. In the MassHunter concept, you will find the setting in the **Method Editor** tab.

Set Error Method	Sets the method that is loaded if an error occurs to the method that is currently available in the hardware.
Identify Device	Causes the LED on the front of the module to blink for a few seconds.
Switch Pump On/Off	Toggles the status of the pump, on or off.
Switch solvent selection Valve A	Allows you to switch the solvent inlet line for channel A from inlet line 1 to 2.
Switch solvent selection Valve B	Allows you to switch the solvent inlet line for channel B from inlet line 1 to 2.
Bottle Fillings	Displays the Bottle Fillings dialog box.
Prepare Pump	Allows you to control the Purge, Condition, or the Prime function. • Purge: Purge the LC pump. Fill the system with fresh or different solvent. Follow the directions for purging the pump in the user guide for your pump. • Conditioning: Condition or equilibrate the column. After you purge the pump, you set up to condition or equilibrate the column. ◦ Enter LC parameters in the Method Editor menu, and click Apply to download the method parameter to the LC or, ◦ To select an LC conditioning method, select one from the Method list at the top of the Data Acquisition window. **NOTE:** Conditioning can also be used to remove micro air bubbles. For this measure you have to use a reasonable flow rate (for example 1.5 mL/min), composition setting (for example A: 50 % B: 50 %) and backpressure (>200 bar) to ensure efficient air bubble removal from all pump heads. For further info, please follow the instruction in the technical note *Best Practices for Using an Agilent LC System*. • Prime: If conditioning for 15 min cannot remove air from the pump heads, the Prime function can help. The module draws 20 times solvent at a high speed with all pump drives simultaneously and dispenses it into the waste position of the automatic purge valve. The Prime function stresses the valve and rotor seal. Therefore, it should be performed only as a last measure, before forcefully filling the pump heads with a syringe or attempting to repair the pump heads.
Flush sample loops	Use the gradient start condition for flushing all 2D sample loops and flush the 2D flow path with the flush gradient defined in the method. **NOTE:** A small amount of the 1D solvent is transferred into the flow path of the second dimension during the switching of the valve in the flushing process.
Seal Wash Prime	Allows you to refill the Seal Wash lines once the seal wash solvent has been changed.

Switch Valve	Allows the selection of different valves e.g. ASM Valve and the change of their valve position
Modify	Allows you to configure/modify the 2D-LC capillaries and the transfer volume.
Modify Capillaries	Displays the Modify Capillaries dialog box. In this window you can configure the sample loop, transfer capillary and ASM capillary, see Configure the 2D-LC Cluster on page 119.

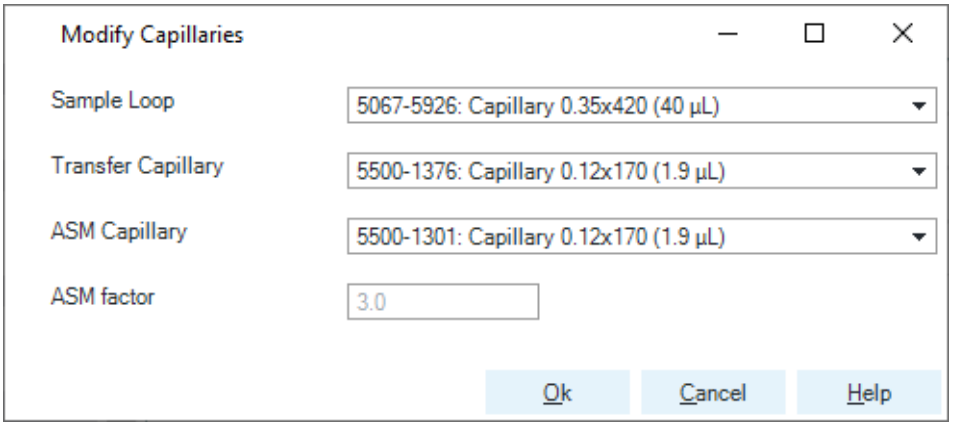


Figure 69: Modify capillaries windows allows the configuration of the sample loop, transfer capillaries, and ASM capillaries

Modify Transfer Volumes	Displays the Modify Transfer Volumes dialog box. In this window, you can configure the transfer volumes for the ^{1}D detector and the ^{2}D detector.

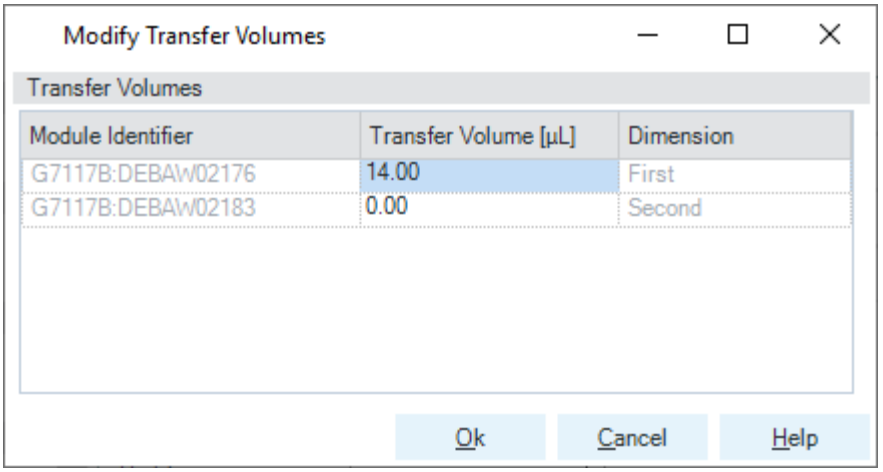


Figure 70: Modify Transfer Volumes windows allows the configuration of the transfer volume

Transfer Volume ^{1}D detector

The ^{1}D settings for the transfer volumes that determine the time between the ^{1}D detection of the peak and the switching of the 2D-LC valve, depend on the hardware setup. For a standard 2D-LC with two DADs, the transfer volume is approx. 14 µL. To calculate the volume, you have to add half the volume of the detector flow cell plus the volume between the detector flow cell and the 2D-LC valve.

NOTE

If a second ^{1}D detector is installed, the transfer volume between the two detectors in the first dimension volume must also be entered in the signal selection of the reference chromatogram see Method Parameters on page 152.

Transfer Volume ^{2}D detector

The transfer volume for the ^{2}D detector defines the volume between the 2D-LC valve and the second dimension detector flow cell.

NOTE

For the MassHunter workflow with a High-End mass spectrometer as additional second detector, you have to define this transfer volume (delay) in the data evaluation.

2D-LC Valves Online Monitor in the 2D-LC User Interface

The Online Monitor displays the status of the 2D-LC valve. The following illustrations show some examples so that you can see what is happening at any time during operation.

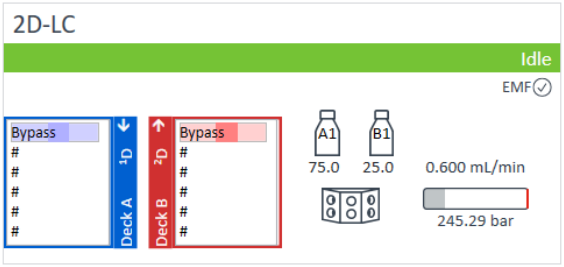


Figure 71: No sampled/parked cuts, mobile phase through loop of each deck (indicated by bypass)

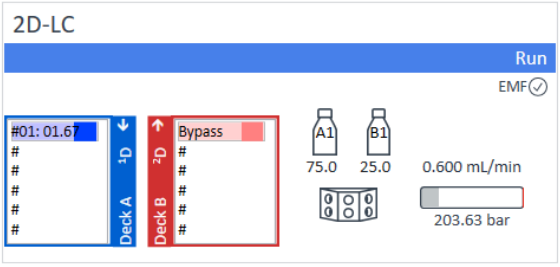


Figure 72: Heart Cut sampling/parking indicated by blue beam moving along, cut number and time in minutes

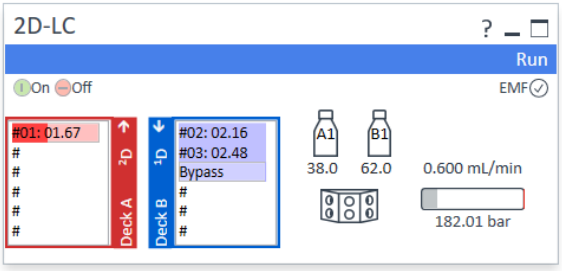


Figure 73: [2]D-analysis indicated by red beam moving along

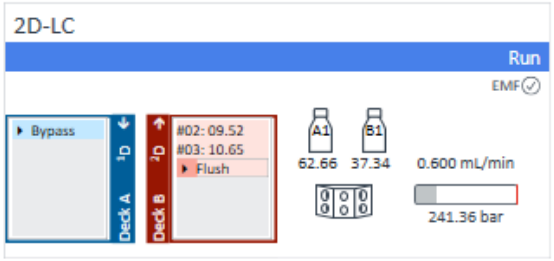


Figure 74: Flush indicated by red beam moving along

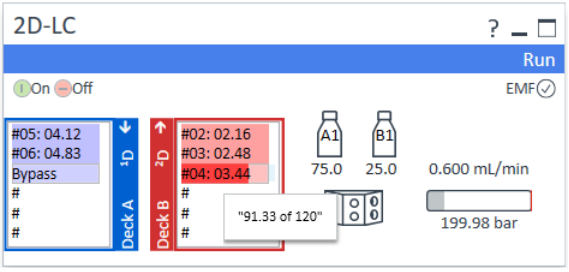


Figure 75: Hovering over analysis loop indicates time passed and time remaining (in seconds)

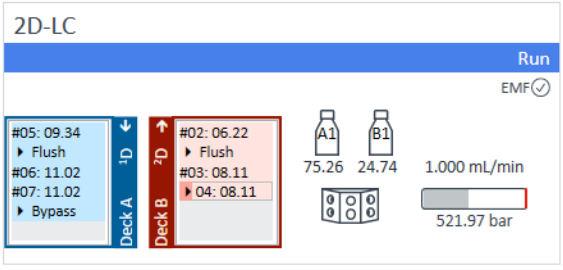


Figure 76: HiRes series give the same parking time (here cuts 3 and 4, and 6 and 7)

Method Editor Window

In the Method Editor window, you enter acquisition parameters for the method, see Method Parameters.

NOTE To decouple the Method Editor from UI, double click the Method Editor bar. Then you can enlarge the window to get a full screen view for programming.

Sample Run Window

In the sample run window, you enter sample information to run individual samples interactively, and you can start a single sample run.

Worklist Window

With the worklist window, you enter sample information for multiple samples.

When you run the worklist, the samples are automatically run in the order listed in the worklist.

You can add one or more tune actions to the Worklist when you add a factory script to the worklist.

Tune Window

In the Tune window, you tune the mass spectrometer. You can use one of the automated tuning algorithms, or you can manually tune the instrument. Manual tuning can result in a less than optimal tune; however, if you perform a manual tune, Agilent recommends that you only manually tune the front part of the instrument: ion source and optics 1. Agilent does not recommend that you manually tune parameters that are after the collision cell.

Instrument Details

In some case, it may be necessary to check the various details such as the firmware and driver version.

The following options to obtain this information exist:

- Use Module List to Obtain Instrument Details on page 144
- Use Instrument Configuration Report to Obtain Instrument Details on page 145

NOTE If an upgrade is needed, see Compatibility Matrix on page 45, or contact your Agilent sales representative.

Use Module List to Obtain Instrument Details

1 Start the Data Acquisition program.

2 Click the **i** Icon in the low right corner of the dashboard.

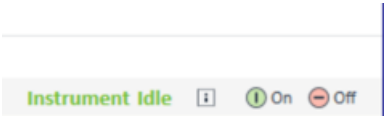


Figure 77: Instrument information view of the dashboard

Module List screen shows up.

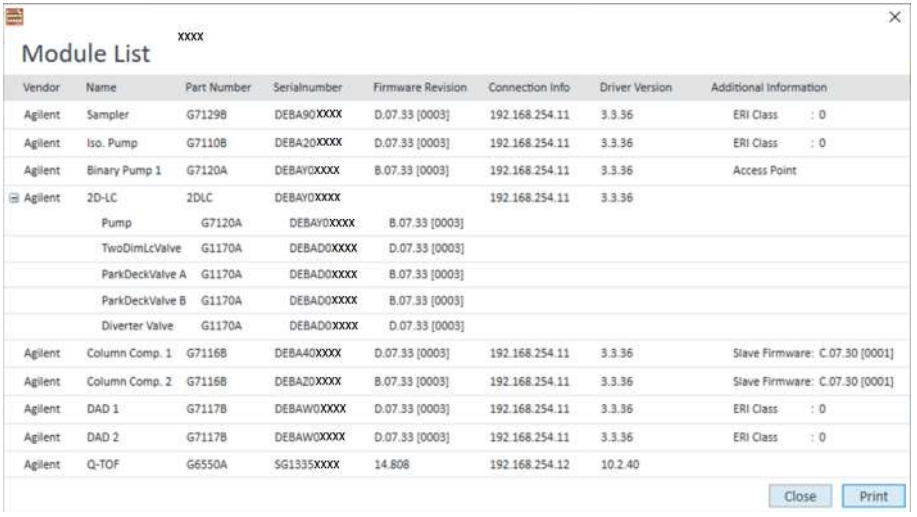


Figure 78: Instrument Module List view using the example of a 2D-LC Q-TOF system

3 Click Print or Close.

Use Instrument Configuration Report to Obtain Instrument Details

1 Start the Data Acquisition program.

2 Select the Instrument Configuration from the **Print Reports** layout.

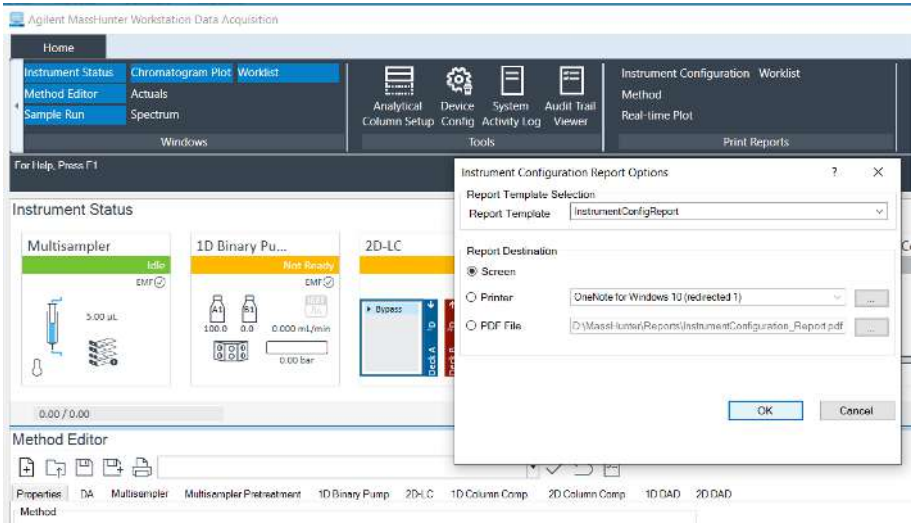
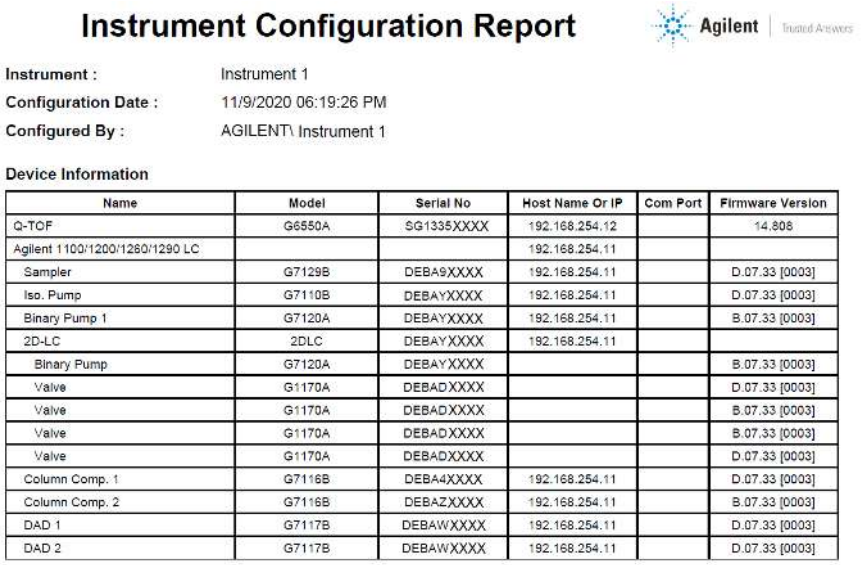


Figure 79: Instrument Configuration Report Option view using the example of a 2D-LC Q-TOF system

3 Click Screen.

4 Click OK.



Instrument Configuration Report

Agilent | Trusted Answers

Instrument : Instrument 1
Configuration Date : 11/9/2020 06:19:26 PM
Configured By : AGILENT\ Instrument 1

Device Information

Name	Model	Serial No	Host Name Or IP	Com Port	Firmware Version
Q-TOF	G6550A	SG1335XXXX	192.168.254.12		14.808
Agilent 1100/1200/1260/1290 LC			192.168.254.11		
Sampler	G7129B	DEBA9XXXX	192.168.254.11		D.07.33 [0003]
Iso. Pump	G7110B	DEBAYXXXX	192.168.254.11		D.07.33 [0003]
Binary Pump 1	G7120A	DEBAYXXXX	192.168.254.11		B.07.33 [0003]
2D-LC	2DLC	DEBAYXXXX	192.168.254.11		
Binary Pump	G7120A	DEBAYXXXX			B.07.33 [0003]
Valve	G1170A	DEBADXXXX			D.07.33 [0003]
Valve	G1170A	DEBADXXXX			B.07.33 [0003]
Valve	G1170A	DEBADXXXX			B.07.33 [0003]
Valve	G1170A	DEBADXXXX			D.07.33 [0003]
Column Comp. 1	G7116B	DEBA4XXXX	192.168.254.11		D.07.33 [0003]
Column Comp. 2	G7116B	DEBAZXXXX	192.168.254.11		B.07.33 [0003]
DAD 1	G7117B	DEBAWXXXX	192.168.254.11		D.07.33 [0003]
DAD 2	G7117B	DEBAWXXXX	192.168.254.11		D.07.33 [0003]

Figure 80: Instrument Configuration Report view for detailed overview of the modules using the example of a 2D-LC Q-TOF system

Logbook in MassHunter Workstation

Sometimes it is necessary to check the processes that take place in a InfinityLab LC/MSD instrument. Therefore, there is a log file in which the processes are logged. This log file provides important data for the analysis of the system.

View the logbook

1 Start the data acquisition via the Control Panel.

2 Select the System Activity Log from the Tools layout will start the Logbook Viewer program.

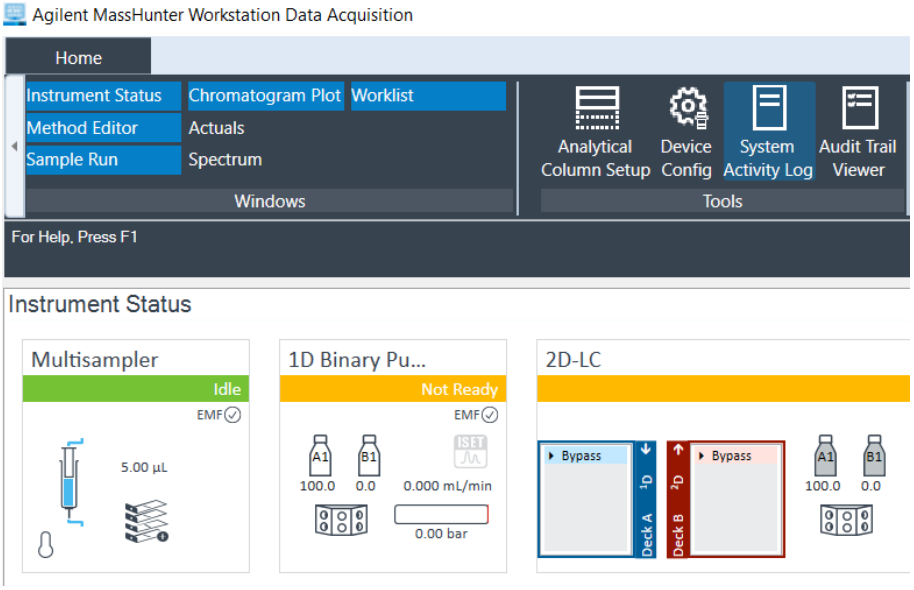


Figure 81: System logbook viewer from MassHunter Workstation Data Acquisition

Configure logbook notification

If you get more logbook notifications than is useful to you, you can change the type of notifications that are displayed.

1 Click on **Filters** in the taskbar.

2 Select the type of notifications that you want displayed.

Figure 82: Activity Log Viewer

3 Click **Search**.

Online Help 2D-LC

1 To get more information about a window or dialog box, place the cursor on the window or dialog box of interest and press F1.

2 From the Help menu, access How-to help and reference help.

Important Customer Web Links

- To access Agilent training and education, visit https://www.agilent.com/chem/training to learn about training options, which include online, classroom and onsite delivery. A training specialist can work directly with you to help determine your best options.
- To access the *Agilent Resource Center* web page, visit https://www.agilent.com/en-us/agilentresources. The following information topics are available:
  - Sample Prep and Containment
  - Chemical Standards
  - Analysis
  - Service and Support
  - Application Workflows
- The *Agilent Community* is an excellent place to get answers, collaborate with others about applications and Agilent products, and find in-depth documents and videos relevant to Agilent technologies. Visit https://community.agilent.com/welcome
- Videos about specific preparation requirements for your instrument can be found by searching the *Agilent YouTube* channel at https://www.youtube.com/user/agilent
- *Need to place a service call?* https://www.agilent.com/en/promotions/flexible-repair-options

6 Method Parameters

This chapter provides background information on method parameters. It helps to optimize methods in 2D-LC Solution in the modes standard heart-cutting, multiple heart-cutting, high-resolution sampling and comprehensive 2D-LC.

Method Editor 2D-LC

The method setup dialog is used to edit the 2D-LC specific method parameters.

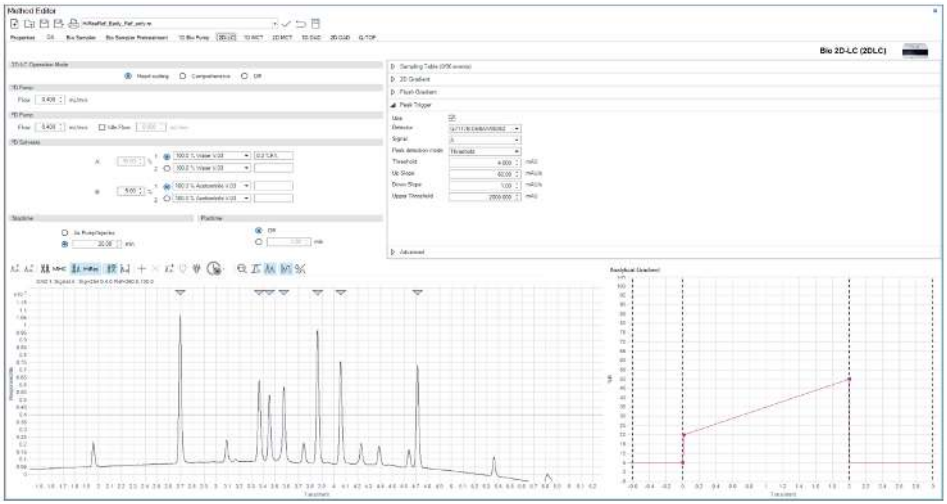


Figure 83: 2D-LC method setup

The setup of following method parameters is available:

- 2D-LC Operation Mode, see 2D LC Operation Mode on page 155
- Solvents, see Define the ^{2}D Solvent on page 158
- Flow settings, see Define the ^{1}D Pump Flow on page 156 and Define the ^{2}D Pump Flow on page 157.
- Stoptime, see Define the Stoptime on page 159
- Posttime, see Define the Posttime on page 161
- Sampling table, see Edit the Sampling Table on page 162
- 2D Gradient, see Define the D Gradient
- Flush Gradient, see Use Flush Gradient on page 177
- Peak Detector Operating values, see Use Peak Trigger on page 178
- Advanced, see Use the Advanced ^{2}D Pump Settings on page 184
- Reference Chromatogram, see Preview (2D-LC) on page 187

- Analytical or Flush Gradient Preview, see Set up Second Dimension Gradient with the Graphical User Interface on page 208

NOTE To get more information, in the software press **F1** that starts the Online Help of the software.

Set the 2D-LC Method parameters

2D LC Operation Mode

Setting the mode has the following consequences:

Heart-Cutting (LC-LC)

The Heart-Cutting mode covers two 2D-LC applications Heart Cutting (LC-LC) and High-Resolution Sampling (HiRes). Once you have selected the Heart-Cutting mode, you can later define in the software whether you want to use one or the other mode or even both together.

In Heart-Cutting modus, a relevant volume of ^{1}D is cut off and injected onto the ^{2}D column using the ^{2}D pump. A peak trigger or a time window defines the volume to be injected on the ^{2}D column. When heart-cutting starts, a loop is filled with the peak of interest. Then the injection on the ^{2}D starts running the gradient of the ^{2}D pump.

For details Setting this mode, see Heart-Cutting 2D-LC (LC-LC) on page 17.

In contrast to Heart-Cutting, which uses the continuous flowthrough principle, in High-Resolution Sampling (HiRes) the Multiple Heart-Cutting (MHC) valve is switched before and after parking the peak.

When setting up the experiments, keep the following general considerations in mind:

- Each loop for consecutive snips stores the same sample volume.
- First and last loop cannot be used for parking.
- Solvent transfer from ^{1}D to ^{2}D can be reduced.
- Cut number 5 cannot be parked entirely in the Sample Loop. Otherwise cut 6 would got partially to the transfer capillary and would therefore be lost or spoil cut 5. Cut 5 stays partially in the transfer line and is immediately being analyzed in ^{2}D.
- For parking cut 6 in the Sample Loop, the cut first needs to be moved from the 2D-LC Valve to the deck valve. This new volume must be defined in the configuration of the 2D-LC system.

For details Setting this mode, see High-Resolution Sampling - Peak Parking Principles on page 24.

Comprehensive 2D-LC (LC*LC)

If you have selected comprehensive 2D-LC, the entire volume of the ^{1}D will be injected (using the ^{2}D pump) onto the ^{2}D column. Two identical loops are used alternating, while one loop is filled in ^{1}D, the volume of the other loop is separated with the ^{2}D column.

The Modulation time reflects the duration of one injection cycle in the ^{2}D. After that time, the solvent composition gradient will be repeated. The parameter Modulation time is only used in the Comprehensive mode. The ^{2}D Gradient stop time reflects the maximal duration of the gradient in ^{2}D; the smallest value is 0.01 min. After that time, the Percent B value before the gradient (or the timetable entry at time = 0.0) is restored. In the Comprehensive 2D-LC mode, the gradient stops latest when the Modulation time is reached.

Off

Setting the mode OFF then the 2D-LC functionality is disabled. The 2D-LC instrument is used as a standard 1D-LC instrument that allows you to carry out a ^{1}D run.

Define the [1]D Pump Flow

1 Set the **[1]D Pump Flow** (recommended range is e.g., for LC-LC: 0 – 1.0 mL/min and for LCxLC: 0 – 0.2 mL/min).

This setting defines the flow in the first dimension being used while 2D-LC is active.

Any changes of the Flow parameter in the 2D-LC UI are automatically synchronized with the Method User Interface of the [1]D pump.

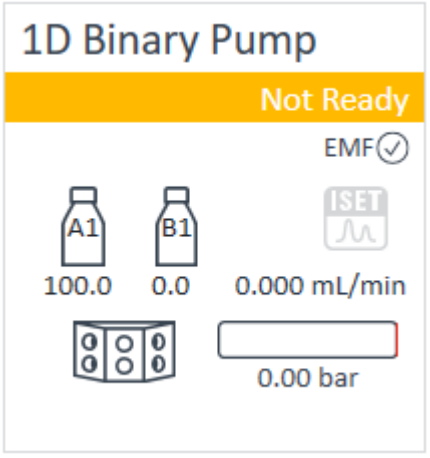


Figure 84: Method User Interface of the [1]D pump

NOTE The selection of the solvents must be done in the standard pump method user interface.

NOTE Maximum recommended [1]D flow rate is 1 mL/min! But this can vary to lower numbers this depends on which 2D-LC operation mode is used (e.g. LCxLC) or to protect the used flow cell for damaging (see flow cell pressure limits).

Define the ^{2}D Pump Flow

1 Set the **^{2}D Pump Flow** (range 0 – 5.0 mL/min).

This setting defines the flow in the 2nd dimension being used while 2D-LC is active (within ^{2}D time segments where mode is not equal to **Off**).

2 To set and use idle flow, select check box **Idle flow**.

The field to define the idle flow is active.

The setting in this field defines the flow in the 2nd dimension that is used while the 2D-LC mode is **Off** (range 0 – 5.0 mL/min) and no cut is analyzed.

NOTE

If **Idle flow** is not selected, the ^{2}D Flow is also used when no ^{2}D analyses take place.

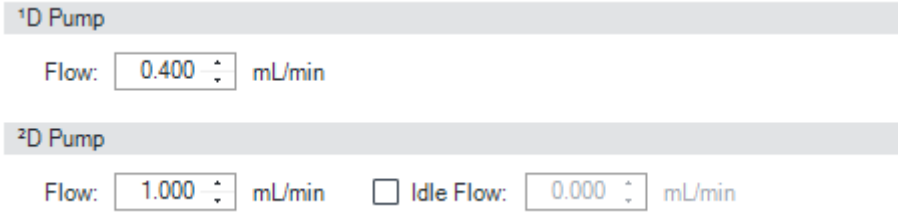


Figure 85: Interface for the flow settings of the ^{1}D pump and ^{2}D pump

Define the ^{2}D Solvent

1 Set the percentage of solvent B to any value from 0 – 100 % in steps of 0.01 %.

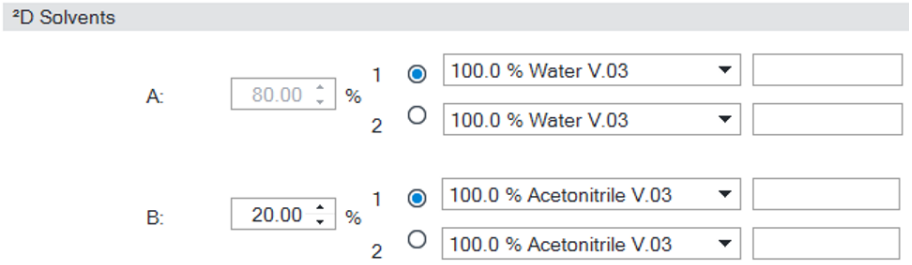


Figure 86: 2D-LC solvent settings

Solvent A always delivers the remaining percentage of volume. If the rate of solvent B is, for example, set to 20 %, solvent A, following the calculation %A = 100 - %B, automatically is set to 80 %. The name of the selected solvents and their solvent channels (A1: ... or A2: ... and B1: ... or B2: ...) are shown in the corresponding text fields.

2 For each solvent, click the down-arrow and select a calibrated solvent from the drop-down list. You can also enter additional information (for example, about buffers) in the adjacent field.

Define the Stoptime

The ^{2}D pump stop time sets a time limit for your 2D-LC measurement. This means the runtime of the 1D run plus the runtimes of all 2D cuts. After the stop time, all gradients are stopped and the pump parameters return to their initial values.

1 To set the stop time, select the radio button and fill in the field **Stoptime**.

NOTE

For the driver-based 2D-LC solution, ensure that the stop time is long enough to include all ^{2}D analyses. The run time will not be extended automatically when cuts remain parked.

NOTE

The ^{2}D pump is the stop time master for the complete 2D-LC system. The stop times of all other modules in the system must be set to **As Pump/ As Injector** except the ^{1}D pump module that should set the Stop Time Modus **As Injector/No Limit**.

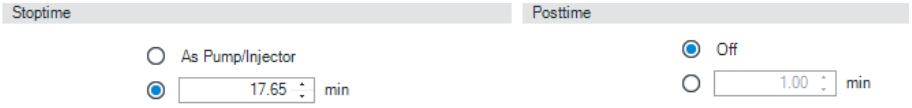


Figure 87: Stoptime and Posttime settings

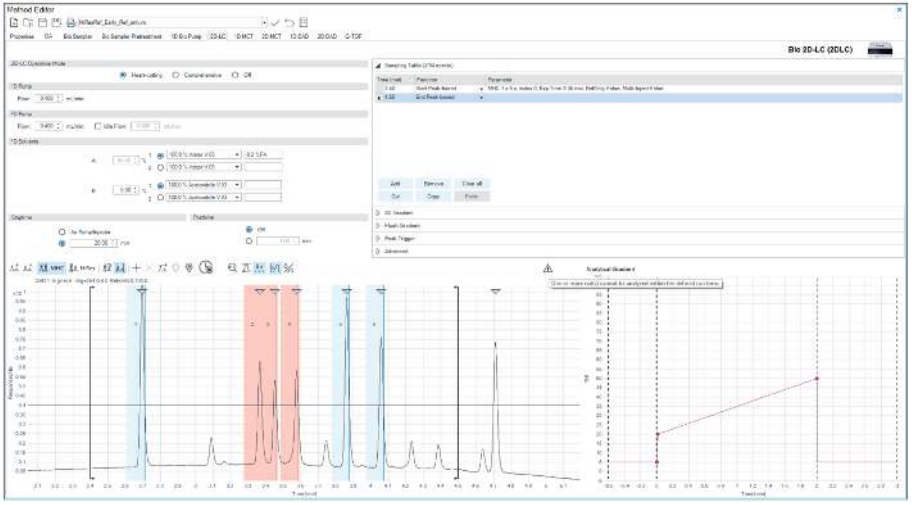


Figure 88: If the stop time is not sufficient for a complete ^{2}D run, a notification triangle pops up

NOTE

If an alert (triangle) pops up in the chromatogram preview you can hover over the sign to get more details. Most likely the stop time of the ^{2}D pump is not sufficient to analyze all ^{2}D cuts see picture above). For a correct stop time alignment click on the stop icon or double click the grey stop time marker in the reference chromatogram. This action will extend the stop time to a valid number.

Define the Posttime

To allow your column to equilibrate after changes in solvent composition (for example after gradient elution), use the post time.

The instrument remains in a post-run state during the post time to delay the start of the next analysis.

1 Check the **Posttime** radio button.

The entry field becomes editable.

2 Specify the post time in the entry field.

Limits: 0.01 – 99999 min.

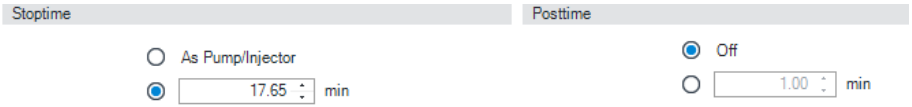


Figure 89: Stoptime and Posttime settings

Edit the Sampling Table

The content of the sampling table specifies when (within the runtime of the first dimension) the selected 2D-LC mode is active.

1 To manually define and edit the sampling table, click one of the buttons:

- Add
- Remove
- Clear all
- Cut
- Copy
- Pause

For example, when you are using the **Add** button a single cut parking event is generated. In this event line you can define the different parameters like time, function, and parameters. Usually for filling the sampling table in time based you are using the **Sample all** feature in the reference chromatogram that generates cuts according to peak detector settings.

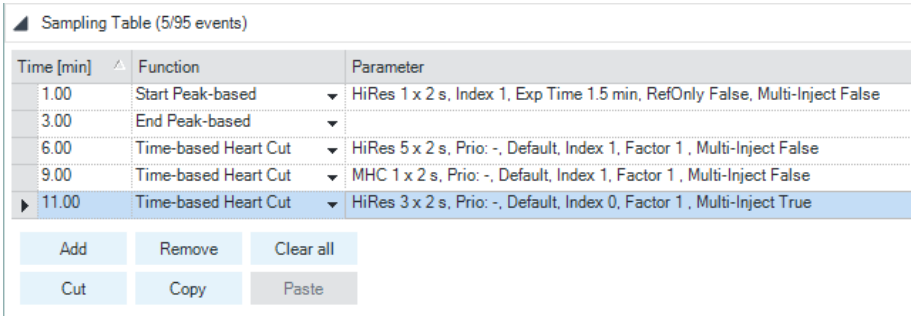


Figure 90: Sampling table for peak-based and multiple heart-cutting events

Table 20: Sampling table description

Type	Description
Time	Defines the start time of the cut.
Function	Defines the mode of sampling. To select an alternative mode, click the down-arrow: • Time-Based Heart-Cuting Define a time-based Heart-cutting run (MHC or HiRes) in the sampling table. • Time-Based Comprehensive Define a time-based Comprehensive run (LCxLC) in the sampling table. • Start Peak-based Define the Start time of a Peak-based Heart-cutting run (MHC or HiRes) in the sampling table. A bracket appears in the preview which marks the Start time of the peak-based area. • End Peak-based Define the End time of a Peak-based Heart-cutting run (MHC or HiRes) in the sampling table. A bracket appears in the preview which marks the End time of the peak-based area. **NOTE:** The selected function in the Sampling Table must match to the 2D-LC Operation mode (Heart-cutting or Comprehensive) to avoid any conflict.

2 In the Sampling Table click in the **Parameter** cell.

Define Parameters for Peak-Based Heart Cut

1 To switch between Multiple Heart-Cutting (**MHC**) and High-Resolution Sampling peak-based (**HiRes**), click the down-arrow.

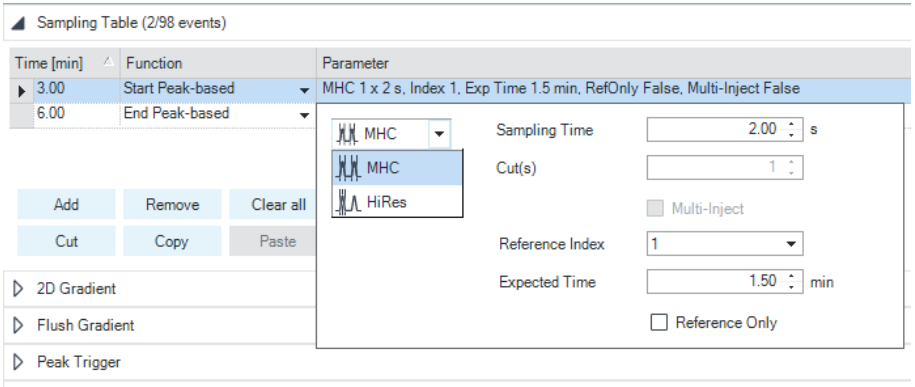


Figure 91: Sampling Table for peak-based MHC and HiRes events

Sampling time (MHC)	The sampling time is the maximal Cut size in seconds (t) in case no peak end is detected by the peak detector. It is calculated from the loop volume (V) / 1D flow rate (F) t=V/F
Cut size (HiRes)	The Cut size in seconds (t) is calculated from the loop volume (V) / ^{1}D flow rate (F) t=V/F. In MHC mode, the cut size for the sample loop is automatically calculated. Therefore the field is unavailable and the number for one cut cannot be changed. In HiRes mode, by default the cut size is automatically calculated for a sample loop filling of 80 %. This calculation reflects the parabolic flow profile of a sample plug in capillaries, which cannot fill a sample loop to 100 %. To get the exact same sample volume in each loop for consecutive snips, the cut size value in HiRes can be changed. t=(V*80 %)/F = 40 µL Sample Loop * 0.8 / 0.6 mL/min ^{1}D flow = 3.2 s
Cut(s)	In MHC mode, only one cut is allowed. Therefore the number of cuts is unavailable. In HiRes mode, you want to get consecutive snips. Therefore the number of cuts can be changed. The maximum number of cuts is 10.
Loop filling	The loop filling factor is unavailable. In MHC mode, the filling factor is read-only and cannot be changed. In HiRes mode, the filling factor is read only but depends on the cut size value.
Multi-Inject	Multi-Inject allows to define a HiRes group to being injected at once, which means the content of the loops is transferred to the ^{2}D column before a single ^{2}D gradient is used for analysis.
Reference Index	Define a Reference Index value for the internal RT-standard (IRTS), which is necessary to use the Dynamic Peak Parking, see Dynamic Peak Parking on page 213.
Expected time	Define the expected time of the internal RT-standard (IRTS).
Reference only	If the checkbox is selected the IRTS will not be analyzed in the second dimension. The IRTS is only detected in the first dimension and the time shift applied to all following time-based cuts.

Define Parameters for Time-Based Heart Cut

1 To switch between Multiple Heart-Cutting (**MHC**) and High-Resolution Sampling (**HiRes**), click the down-arrow.

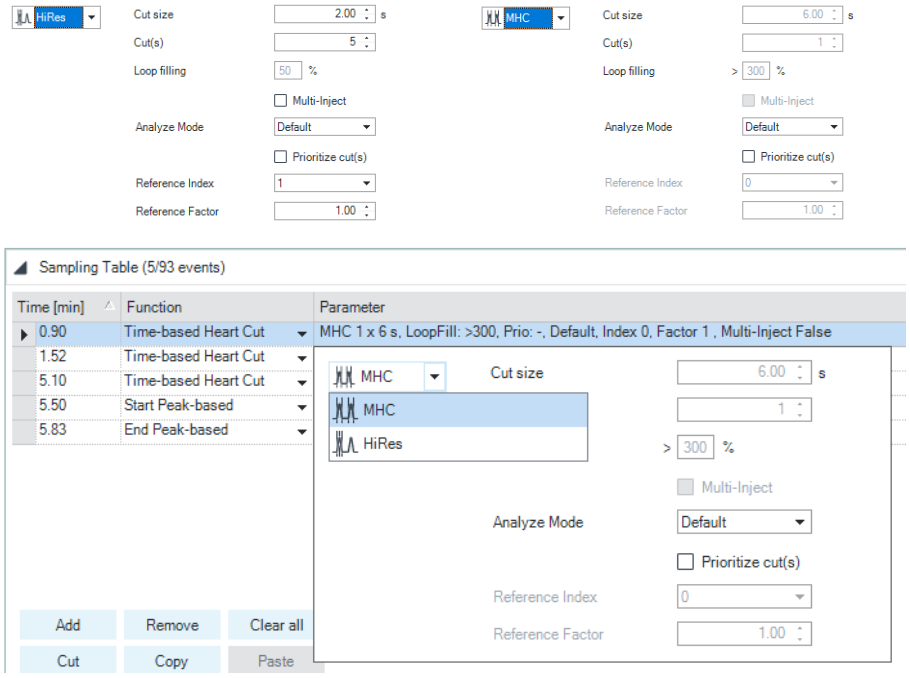


Figure 92: Parameter window in the sampling table for High-Resolution Sampling (HiRes) settings and Multiple Heart-Cutting Settings (MHC)

NOTE If you use the optimization function in the reference chromatogram the analyze mode in the sampling table can be changed automatically

NOTE It is possible to combine MHC and HiRes measurements in one single 2D-LC run.

2 To choose an analyze mode, click the down-arrow and open from the drop-down list.

- Selecting default:

  The cut is analyzed as soon as possible.
- Selecting Delayed:

Analysis is delayed until there is an available time slot.

- Selecting Ignored:

  The cut is not analyzed.

NOTE

If you use the optimization function in the reference chromatogram, the analyze mode in the sampling table can be changed automatically.

3 To specify that analysis of one or more cuts should be given priority, mark the **Prioritize cut(s)** check box.

Define Parameters for Time-Based Comprehensive

Parameters for Time-Based Comprehensive

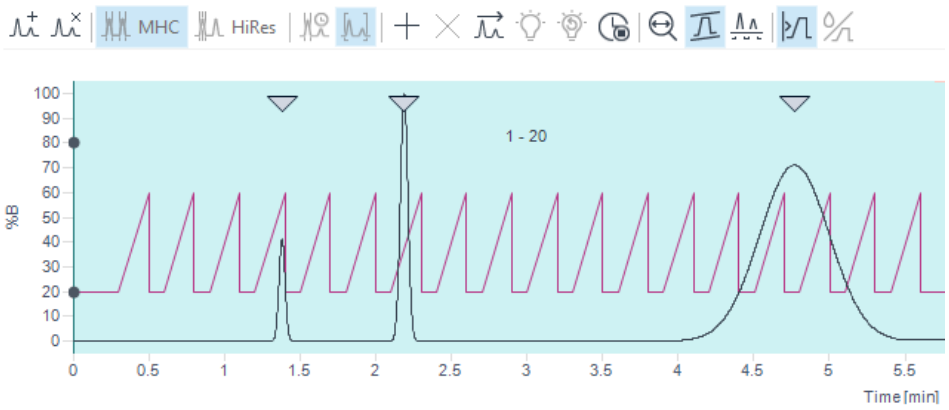


Figure 93: Comprehensive preview with a modulation time 0.3 min (=20 cycles)

1 Enter an absolute time range where the system creates equidistant cuts.

Sampling Table (1/97 events)

Time [min]	Function	Parameter
1.20	Time Based Comprehensive	Comprehensive Range, stop sampling at 10.00 min

Figure 94: Sampling Table time range settings

Comprehensive run starts at the given time.

The modulation time determines the cut size.

2 Enter the stop time of the comprehensive measurement.

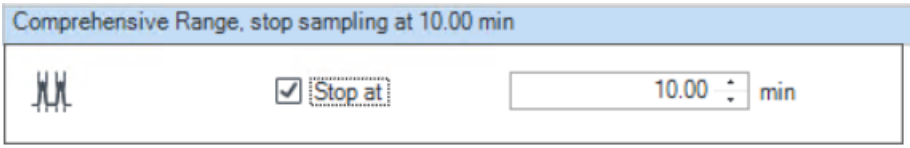


Figure 95: Comprehensive Range settings

Comprehensive Range Stop at, e.g., 10.0 min.

The stop time should coincide with that of the ^{2}D pump.

NOTE In comprehensive the function of the flush gradient is not available.

NOTE If the sampling table is empty, no 2D-LC operation will be executed at all.

Define the ^{2}D Gradient

The 2D Gradient window summarizes all the important settings needed to optimize the gradient method for a second dimension run.

Specify the Gradient Phase

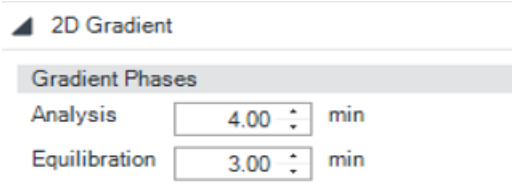


Figure 96: 2D Gradient Phases view

1 Specify the duration (in minutes) of the ^{2}D run for a single cut in the **Analysis** field.

2 To stabilize the system for the next ^{2}D run, specify equilibration time in min in the **Equilibration** field.

The sum of the analysis time and equilibration time is the 2D-LC cycle time, which is shown in the Modulation (2D-LC ^{2}D Gradient) section.

The values in the ^{2}D Gradient Phase are synchronized with the Analytical Gradient display at the bottom right of the screen.

NOTE Different start conditions in the first row may cause step gradients and RI-effects (density differences of the different liquid phases may cause different DAD detection through baseline disturbances).

NOTE When selecting the parameters in comprehensive mode, always consider the modulation time and the loop filling state. To completely transfer the content to the second dimension, do not exceed the filling status of 80 %.

Use Loop Flushing and Active Solvent Modulation (ASM)

If your 2D-LC instrument is equipped with the G4236A 2D-LC ASM Valve, this method development feature helps finding the optimal dilution of ^{1}D solvents in the sample loop. ASM leads to best ^{2}D resolution at lowest cycle time.

ASM settings of 2D-LC method parameters allow switching on and off the use of the ASM functionality.

- If this option is off, it works as a standard 2D-LC valve without dilution.
- If this option is on, the user can set how often he wants to flush the sample loop during the ASM phase.

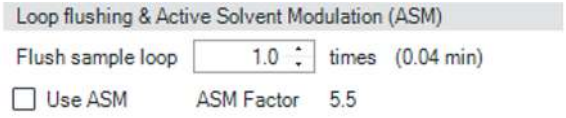


Figure 97: Loop flushing and Active Solvent Modulation settings

1 To use ASM, mark the **ASM** check box.

NOTE

For visual verification of the ASM phase, you can check the Analytical Gradient Graph. There you can see the impact of the ASM phase before the ^{2}D run starts. The gradient with ASM increases the cycle/modulation time.

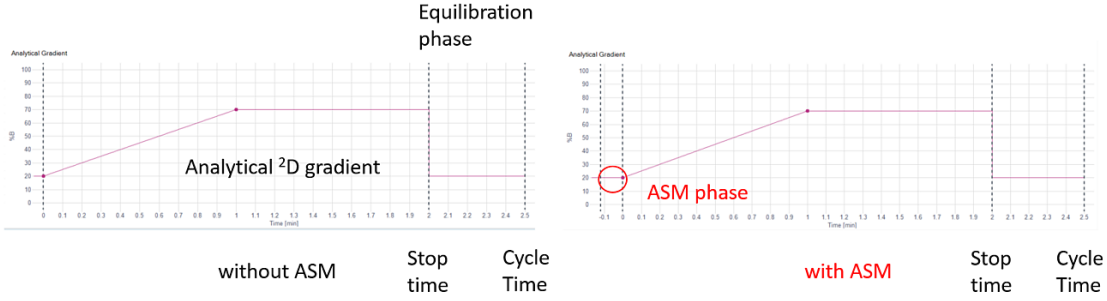


Figure 98: Comparison of analytical gradient with and without ASM phase

This action activates the Active Solvent Modulation.

The ASM Factor is a read-only value and cannot be changed.

NOTE To change the ASM factor you must install and configure the new ASM capillary in the 2D-LC UI, see 2D-LC Capillaries Configuration Tool on page 313.
There are four different ASM capillaries available. You can find more info for Installation and Configure of different ASM capillaries for optimizing the results, see Connecting the 2D-LC Valve, ASM (G4243A) on page 65.

2 Specify the number of times to flush the sample loop in the Flush Sample Loop field.

The total flush time is calculated and displayed.

NOTE Flushing the sample loop three times is typically enough and the recommended default. Less time may be sufficient and can be verified during optimization. The user interface displays how long the flushing will take.

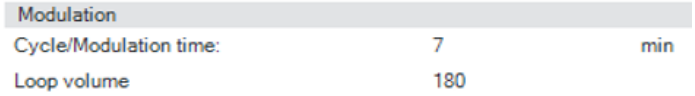
NOTE When using the Active Solvent Modulation (ASM), the valve cycle has four switches - twice as many as for standard 2D-LC valve. More switches per injection affect the lifetime of the rotor seal and must be respected for maintenance intervals.

Modulation

The Modulation section shows the 2D-LC Cycle/Modulation time, which is the sum of the analysis time and the equilibration time specified in the Gradient Phases section.

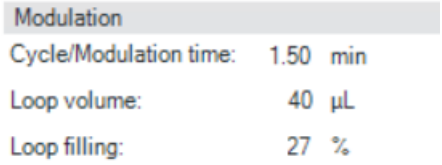
The modulation time also depends on the sample loop built into the instrument, see Recommendations for Instrument Setup on page 57. These values are read-only and cannot be edited.

Heart cutting



Cyle/Modulation time	The cycle time reflects the duration of an LC-LC injection cycle in the second dimension. After that time, the solvent composition gradient for the next cut will be repeated.
Loop volume	The Loop volume represents the configured sample loop volume.

NOTE The info of the loop filling in heart cutting is displayed in the sampling table.

Comprehensive	
Cycle/Modulation time	The Modulation time reflects the duration of one LCxLC injection cycle in the second dimension. After that time, the solvent composition gradient will be repeated.
Loop volume	Loop volume represents the configured sample loop volume.
Loop filling	Loop filling represents the actual loop filling value.

NOTE If the loop filling for LCxLC is smaller than 20 % or higher than 80 %, a notification triangle will be displayed.

NOTE The optimal percentage of the 2D column volume filled by the injection volume (loop filling (%) of the sample loop) is smaller than 10 %.

Specify the Switch Time of the Diverter Valve

The diverter valve can be used to automatically divert salt or buffers coming from the ^{1}D mobile phase to waste at the beginning of every ^{2}D analysis.

This section is active only if a diverter valve is included in the 2D-LC Cluster configuration.

Figure 99: View of an installed Diverter Valve

1 To turn on switching of the diverter valve, mark the check box **Use Diverter Valve**.

The **Switch time** field becomes active.

2 Specify a switch time in the **Switch time** field.

The valve is switched to the detector at the specified time after the start of the ^{2}D analysis and switched back to waste when the ^{2}D analysis has finished.

Set up the Gradient Time Table for the Analytical Gradient

Use this section to set up the eluent gradient timetable for the ^{2}D analysis.

1 Specify the time for the change of solvent composition in the **Time[min]** field.

NOTE

The initial start composition is defined in the ^{2}D solvents table.

2 Specify the percentage of solvent that channel B delivers at the specified time in the **B[%]** field.

Channel A always delivers the remaining volume, %A = (100 - %B). The solvent composition changes linearly from one setpoint to the next.

3 To remove the checkmark in the **Shift** box, select the single Analytical Gradient Event first and then clear the corresponding settings in the **Gradient Shift ^{1}D Time** table.

4 To manually define and edit the **Analytical Gradient**, click one of the following buttons:

- Add
- Remove
- Clear All
- Cut

- Copy
- Paste

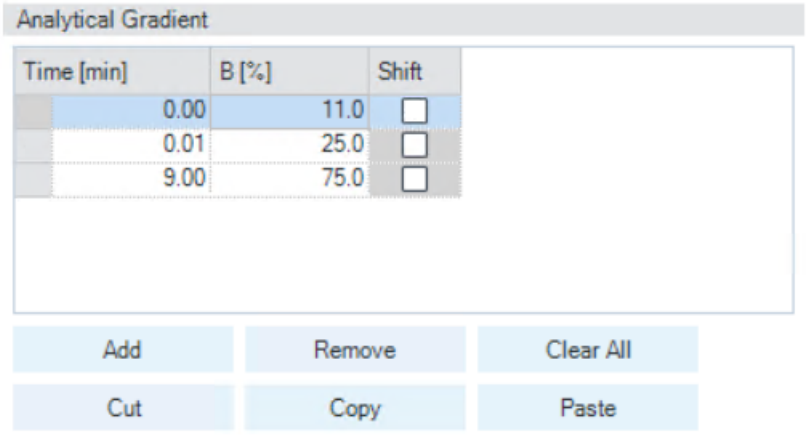
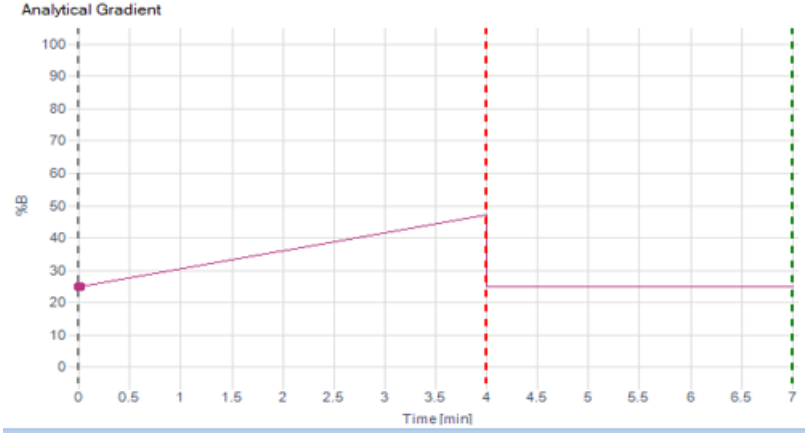


Figure 100: Analytical Gradient Table view

Clicking, e.g., the **Add** button, generates a single analytical gradient event, where you can define the time for the change and the solvent composition.

NOTE To visually verify the Analytical Gradient, check the Analytical Gradient Graph.

NOTE Setup Analytical Gradient can also be done graphically in the preview. The analytical gradient is displayed in purple.



Modify the Solvent Composition in the ^{2}D Gradient Over the Run Time of ^{1}D

Use this section to modify the solvent composition in the ^{2}D gradient over the run time of the first dimension. For each setpoint in the **Analytical Gradient** table that is marked in the Shift column, you can set up a gradient shift as a nested gradient. The gradient shift is used to align the ^{2}D gradient composition with the ^{1}D gradient composition.

Use the table to set up the shifted ^{2}D gradient

1 To set up the shift gradient, select the corresponding line in the **Analytical Gradient** table.

2 Specify the time for the change of solvent composition in the **Time[min]** field.

The shifted gradient composition changes linearly from one setpoint to the next. Change the solvent composition at a specified time. The time axis relates to the stop time of the ^{2}D pump, a time greater than stop time ^{2}D will be ignored.

3 Specify Percent B ranges from 0 – 100 % in the **B[%]** field.

Change the solvent composition at a specified time. Channel A always delivers the remaining volume, %A = (100 - %B). The solvent composition changes linearly from one setpoint to the next.

NOTE Different start conditions in the first row may cause step gradients and RI-effects (density differences of the different liquid phases may cause different DAD detection through baseline disturbances).

NOTE The selected shift check box in the analytical gradient window can only be deactivated by removing the corresponding event in the Gradient Shift ^{1}D Time window.

4 To manually define and edit the shifted ^{2}D gradient, click one of the following buttons:

- Add
- Remove
- Clear All
- Cut

- Copy
- Paste

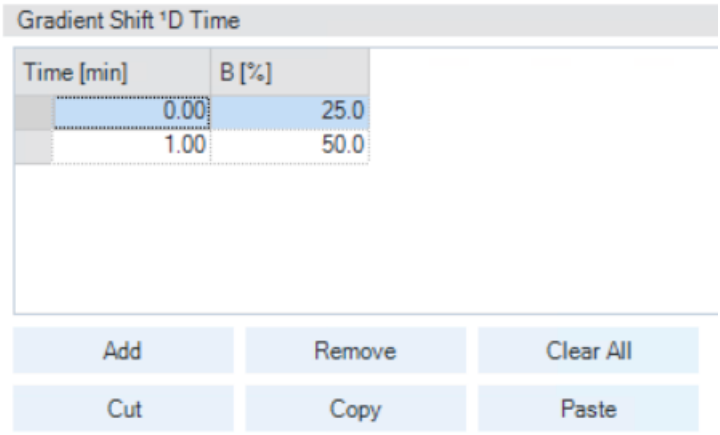


Figure 101: Gradient Shift [1]D Time table

Clicking, e.g., the **Add** button, generates a single gradient shift event, where you can define the time for the change and the solvent composition.

NOTE Set up [2]D Gradient shift can also be done graphically in the preview.

Use Flush Gradient

The **Flush Gradient** can be used to flush the transfer capillaries and Sample Loops. You can choose to use the analytical gradient or you can set up a custom flush gradient. If a flush is required, it is automatically calculated by the system. If you choose **Use custom gradient** option, specify a gradient **Duration**. The **Equilibration** time is the same as that set in the Gradient Phase section.

1 To use the analytical gradient as flush gradient, select the radio button **Use analytical gradient as flush gradient**.

OR: To customize a flush gradient, select the radio button **Customize flush gradient** and use the table to set up the custom gradient:

a Specify the duration time in the Duration field. The equilibration value is read only and is defined in the Gradient Phase settings, see Specify the Gradient Phase on page 168.

b Specify the time for the change of solvent composition in the Time [min] field.

c Specify the percentage of solvent that channel B delivers at the specified time. Channel A always delivers the remaining volume, %A = (100 - %B). The solvent composition changes linearly from one setpoint to the next.

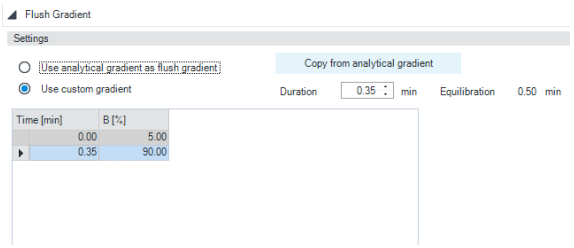


Figure 102: Flush Gradient table view

NOTE **Flush Gradient** is only be used in heart cutting mode. If you have selected comprehensive, this feature is unavailable.

NOTE Setup **Flush Gradient** can also be done graphically in the preview. The **Flush Gradient** is displayed in orange.

Use Peak Trigger

Set Peak Trigger in Time-Based Mode

If the **Use** check box is selected, the peak detection settings are used for finding and marking ^{1}D peaks within the reference chromatogram in the preview UI. This means that first a known ^{1}D reference chromatogram of the instrument must be loaded and then can be used to detect the correct position of sample peaks for a complete 2D-LC measurement.

1 The found cuts are displayed in grey triangles in the preview of the reference chromatogram, see reference chromatogram.

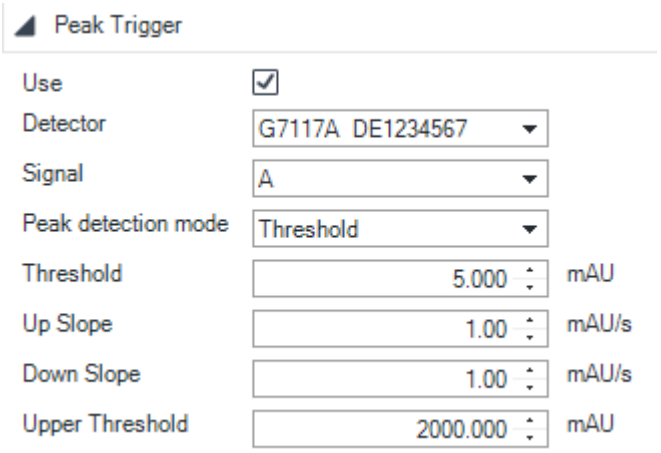


Figure 103: Peak Trigger view

Set Peak Trigger in Peak-Based Mode

If the **Use** check box is selected, the peak trigger settings are used to trigger the sampling and parking of cuts from the first dimension. This means that the areas of interest must be predefined by the method (See sampling table). If a peak appears in the ^{1}D detector and the threshold (or slope) is reached, the 2D-LC modulator starts sampling then the found peaks are parked and analyzed in the second dimension.

1 To enable/disable the peak trigger of the [1]D detector, mark the **Use** check box.

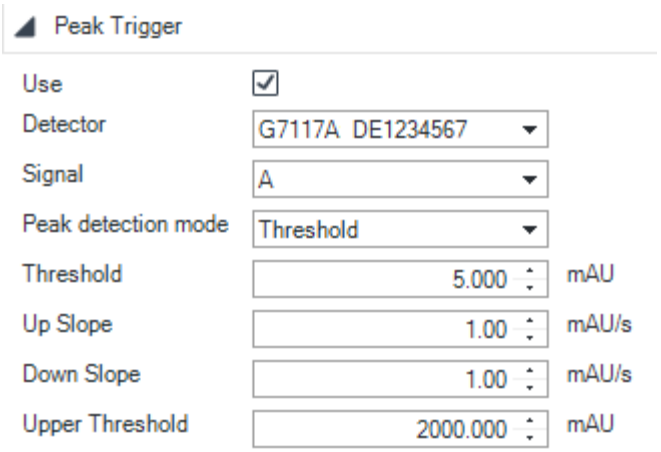


Figure 104: Peak Trigger view

In time-based mode, depending on the **Peak Trigger** settings, peaks can be marked in the preview of the loaded reference chromatogram.

In peak-based mode, the **Peak Trigger** settings can be used for online peak-triggered 2D-LC operation.

2 Select the peak trigger **Detector** from the drop-down list.

3 Select the signal for the peak-based mode from the **Peak detection mode** drop-down list.

4 Select **Threshold**, **Slope** or **Threshold and Slope** from the **Peak detection mode** drop-down list.

a Optional: Set **Threshold**.

In **Threshold** mode, the 2D-LC Valve is triggered on the threshold of the detector signal. The threshold value is given as mAU value. When the UV signal rises above this value, with a certain delay the 2D-LC Valve is triggered and switches to cut the fraction. The 2D-LC Valve will switch to the next position when the UV signal falls below this value or the cut size elapse.

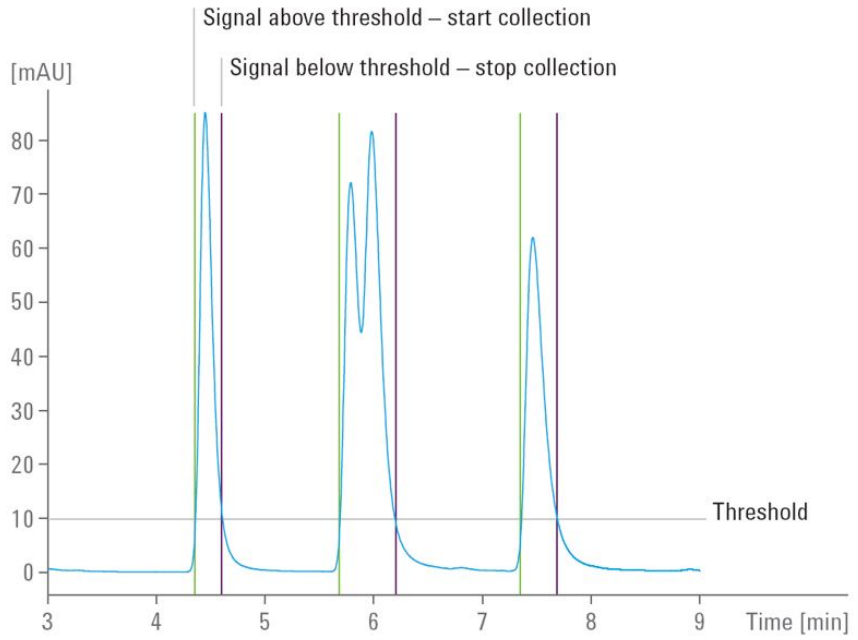


Figure 105: Chromatogram with Threshold line

b Optional: Or set the **Slope**.

In **Slope** mode, the 2D-LC Valve is triggered on the slope of the detector signal. Adequate values for Up Slope and Down Slope can be specified in the corresponding fields. This value is given as mAU/second. The 2D-LC Valve

switches when the up slope exceeds the given value. Cutting ends when the slope passes a minimum and then rises above the down slope value or the cut size is elapsed.

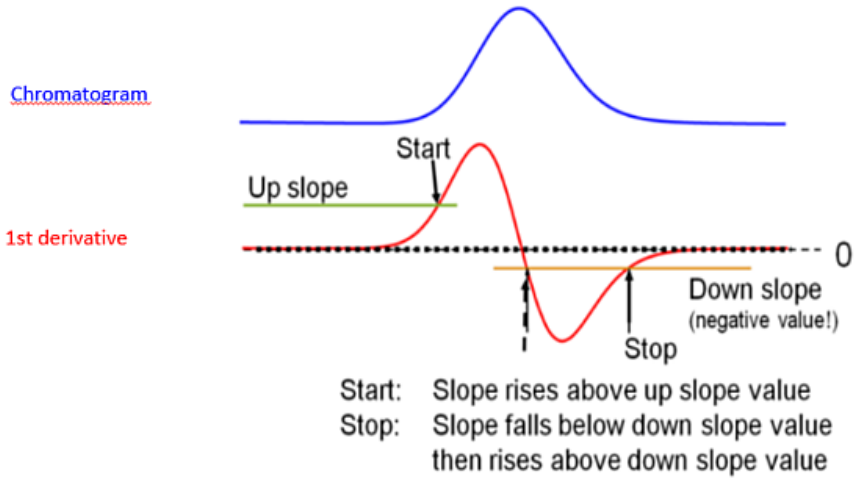


Figure 106: Example for triggering the slope of the detector

c Optional: Or set Threshold and Slope.

In Threshold and Slope mode, the 2D-LC cutting (peak parking) is triggered when the corresponding values for threshold and slope are reached. If the detector signal exceeds both the threshold and the Up Slope value, the cutting of the fraction is started. If the detector signal drops either below the Threshold or the Down Slope value, the 2D-LC Valve stops cutting the fraction by switching the valve to the next position.

For more complex problems, like two overlapping peaks, it is possible to combine slope and threshold collection. The two peaks will be split in two cuts roughly around the local minimum between the two maxima.

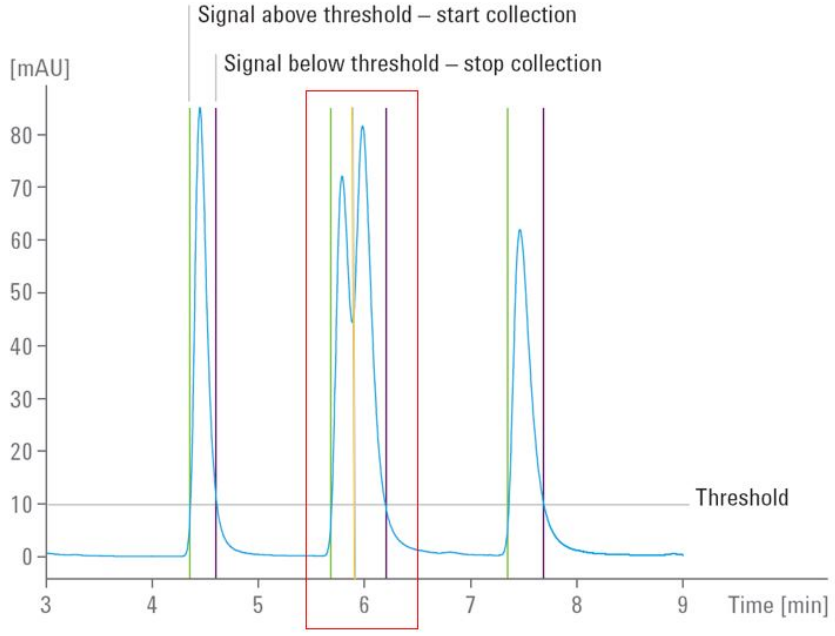


Figure 107: Chromatogram with Threshold and Slope settings

- Threshold

  This method detects peaks based on threshold values only. The height of the peak triggers the peak cutting. The default value is 20.000 mAu
- Up Slope

  This method detects peaks based on up slope values only. The slope of the rising peak triggers the peak cutting. The slope value is based on the first derivative of the signal. The default value is 1.00 mAu/s.
- Down Slope

  This method detects peaks based on down slope values only. The slope of the falling peak triggers the peak cutting. The slope value is based on the first derivative of the signal. The default value is 1.00 mAu/s.
- Upper Threshold

This method detects peaks based on upper threshold values only. The height of the peak ensures that collection is not switched off, even for a saturated signal that might be expected to do so. When the UV signal exceeds the upper threshold, slope collection will be disabled. The default value is 2000.000 mAu.

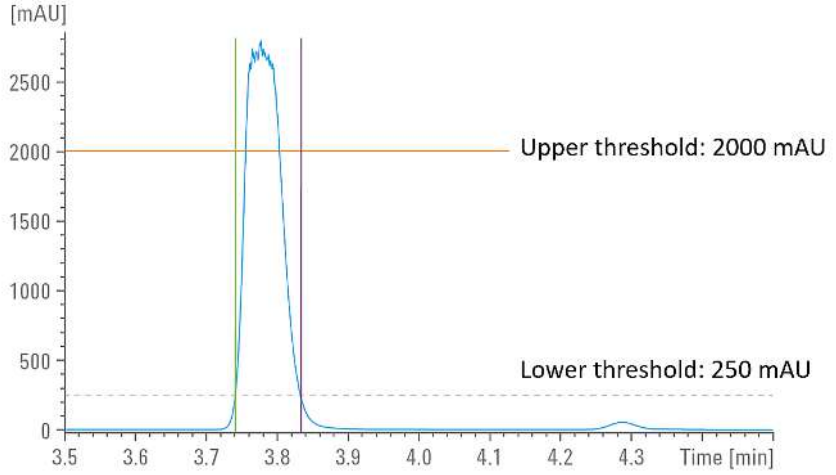


Figure 108: Chromatogram with upper and lower threshold

NOTE

The 2D-LC Valve switches either:

- If the sampling time / cut size has elapsed (sampling time controls cut position), or
- If the signal falls below the threshold or slope (peak-based)

⇨ whichever comes first

Use the Advanced ^{2}D Pump Settings

Advanced settings open the pump method viewlet for Advanced ^{2}D pump settings.

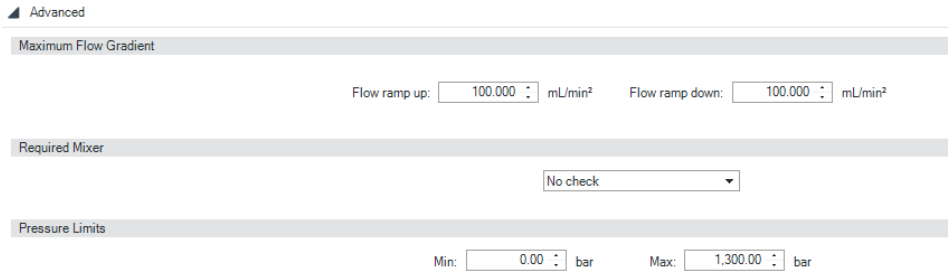


Figure 109: Advanced ^{2}D pump settings

Use the table to set up the additional ^{2}D pump parameters:

1 Set the Maximum Flow Gradient.

You can set a limit on the rate of change of the solvent flow to protect your analytical column.

For the G4220A/B Binary Pumps and G7120A High Speed Pump, you can set individual values for Flow ramp up and Flow ramp down.

2 **Optional:** Select the Required Mixer.

If a mixer is required for the analysis,

- Click the down arrow, and
- Select the required mixer from the drop-down list.

If no mixer is required for the analysis,

- Select **No check** from the drop-down list.

NOTE If a specific mixer is selected, and a different mixer (or no mixer) is detected, the pump stays in a Not ready condition.

3 **Optional:** Set the maximum and minimum **Pressure Limits** for the pump.

NOTE The default settings are recommended. Change these settings only for important and valid reasons.

- **Max** is the maximum pressure limit at which the pump will switch itself off.

  This maximum pressure limit protects the system against overpressure.

- **Min** is the minimum pressure limit at which the pump will switch itself off. This situation can occur, when a solvent reservoir is empty.

  The minimum pressure limit protects the system from damage caused by pumping air.

NOTE For further details, especially the pressure limits, see the user manual of your pump.

Preview (2D-LC)

The Preview panel shows loaded reference chromatogram and the 2D-LC gradient profiles in one or two windows:

- The main window, which is always visible, can show the detector signal of the reference chromatogram and the ^{2}D gradient profile over the whole run. It also allows interactive editing of the cuts with the help of the toolbar.
- The right window, which can be toggled on and off, shows either the ^{2}D gradient profile or the flush gradient profile.

Both gradient profiles can be edited interactively. The Preview is synchronized with the ^{2}D method parameters so that any changes you make in the Preview are also updated in the parameters, and vice versa.

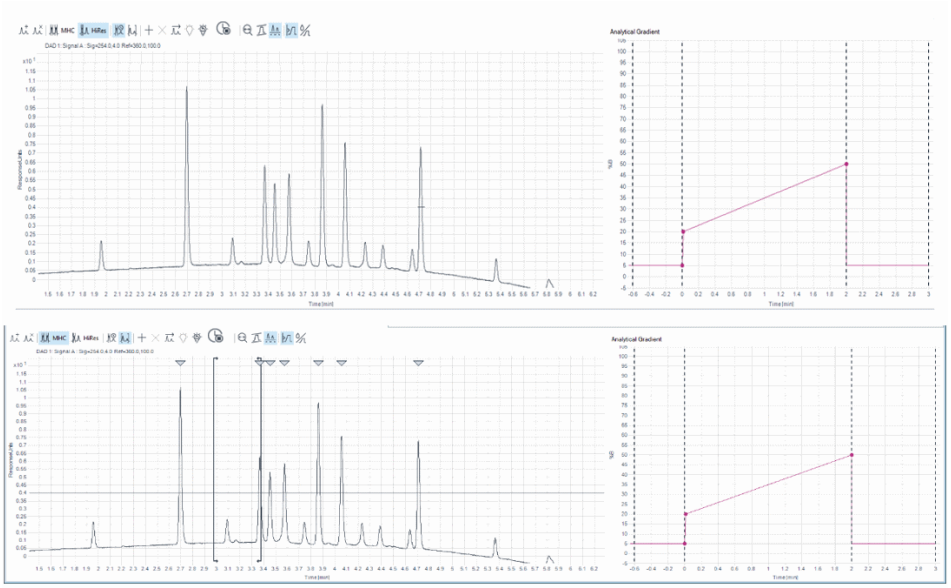


Figure 110: Preview panel with loaded reference chromatogram (top) and with threshold settings and detected peaks in the loaded reference chromatogram (bottom)

To edit and modify the ^{2}D method parameters graphically, the following tools are available in the toolbar:

	Displays a data file selection box that allows you in the next steps to select a [1]D data file.

Figure 111: Selection drop-down menu to select shift volume or shift time

Finally loaded the data file that can be used to display a [1]D reference chromatogram in the Preview.

Uploading a reference signal into the method screen can be helpful to illustrate, at which positions of the chromatogram which cuts will be taken.

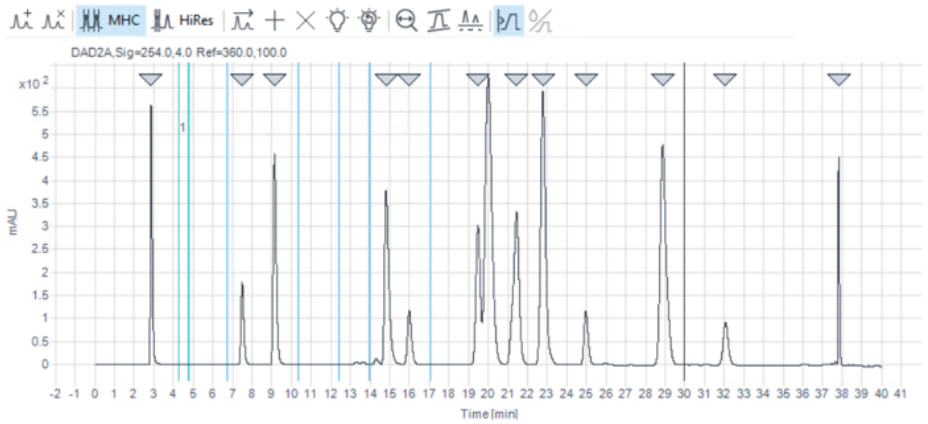


Figure 112: Loaded [1]D chromatogram in preview window

NOTE To cut the peaks correctly, the conditions, such as the flow under which the reference chromatogram was recorded, must be maintained.

NOTE If further [1]D detectors are used as reference chromatogram, the transfer volume must be corrected for these detectors. To do so, enter the shift volume or the shift time.
If only one [1]D detector is configured, the transfer volume is already defined in the 2D-LC cluster and therefore does not need to be corrected here.

NOTE Shift time/Shift volume allows correction between cutting time at valve and detection time. This correction might be necessary if the tranfer volume which is defined in the 2D-LC cluster will not fit to the loaded ref chromatogram in the 2D-LC cluster (see page 2D-LC Cluster). An example is the Switchable [1]D/[2]D Setup where [1]D mass spectrometry data are used as reference chromatogram.

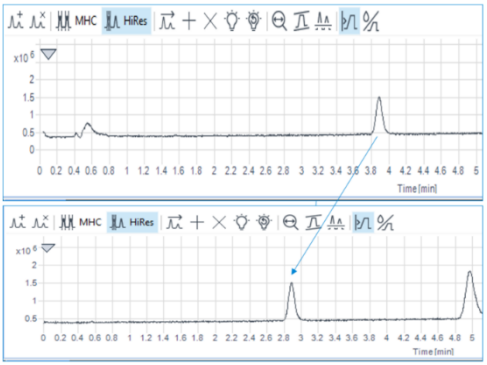
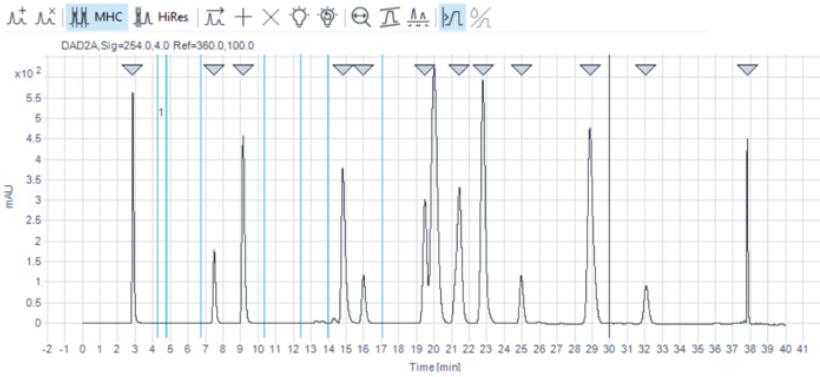


Figure 113: Example of a loaded Q-TOF signal with shift volume set to 0 µL compared Q-TOF signal with shift volume set to -100 µL

	This tool removes the current reference chromatogram from the Preview.
	This tool switches to Multiple Heart-Cutting mode (**MHC**). This tool can only be used if heart-cutting is selected in the 2D-LC Operation mode. The function is used with the Add/Delete or Sample all function.
	This tool switches to High-Resolution Sampling mode (**HiRes**). This tool can only be used if heart-cutting is selected in the 2D-LC Operation mode. The function is used with the Add/Delete or Sample all function.
	This tool switches to Peak-based mode.
	This tool switches to Time-based mode.

NOTE In one 2D run the **MHC** and **HiRes** modes can now be combined.

	Using this tool, depending which mode **MHC** or **HiRes** is selected, will automatically sample the entire chromatogram using the current Peak Trigger parameters and enters all detected cuts into the Sampling Table. This tool can only be used if the use box in the peak trigger section is checked. In the reference chromatogram, gray triangles show all detected peaks. 
	This tool allows you to add a single cut manually. The single cut will be displayed in the reference chromatogram and in the sampling table. **NOTE:** Double click the **+** tool leads to permanent activation of this function. Cancel this activation by repeated double-clicking the **+** tool.
	To remove a single cut from the reference chromatogram and the sampling table, mark the single cut and then click the tool. **NOTE:** Another method to add or remove a cut is using the right mouse button to **Add Cut** or **Delete Selected Cut**. It is also possible to mark the cut or mark a line and press **Del** on the keyboard.
	This feature allows you to optimize the parking of cuts so that the highest number can be analyzed in the available time. The Sampling Table is updated to show which cuts have been allocated a delayed analysis. Smart peak parking optimizes parking for all time-based peaks in a reference signal. Optimizing Goals: • Capture as many peaks as possible and, if necessary, extend the run time • Analyze peaks as fast as possible.

If still some peaks cannot be parked, user can define important peaks (Prioritize) in the sampling table.

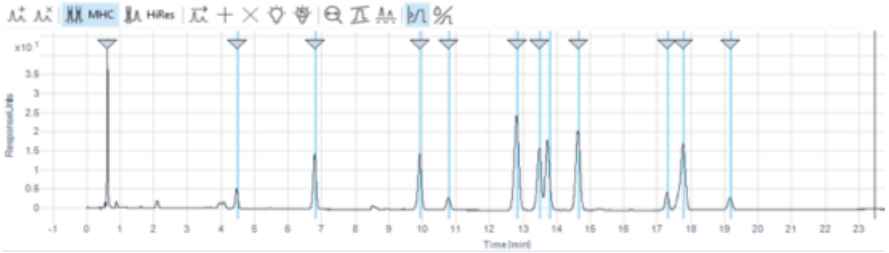


Figure 114: 2D run optimization done all peaks (blue) are analyzed compared to without optimization below

	This tool resets the current optimization, disables smart parking, but it will keep the run time extension.
	The tool adjusts the stop time to the real run time. The same task can be achieved by double-clicking the vertical stop line in the preview.
	This tool resets all zoomed graphics to their normal magnification. Zoom out. For zooming in, press the left mouse button and drag over the desired area to be zoomed. **NOTE:** To zoom out step by step, double-click once with the left mouse button.
	This tool switches the display of the gradient in the preview on or off. This function overlays the gradient at a glance in a complete run. For manually changing the gradient setting in the preview, see Set up the Gradient Time Table for the Analytical Gradient on page 173.

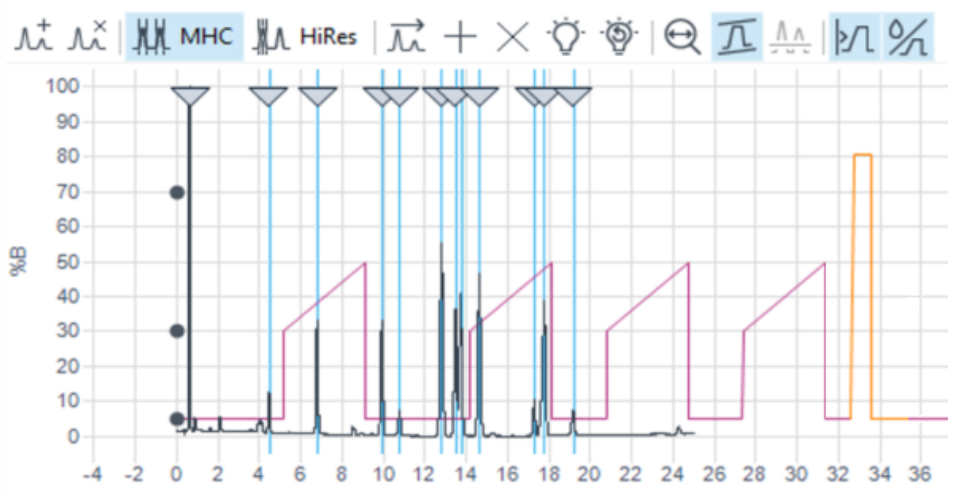
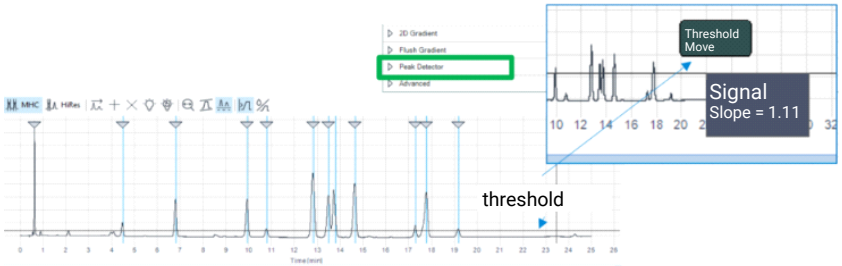


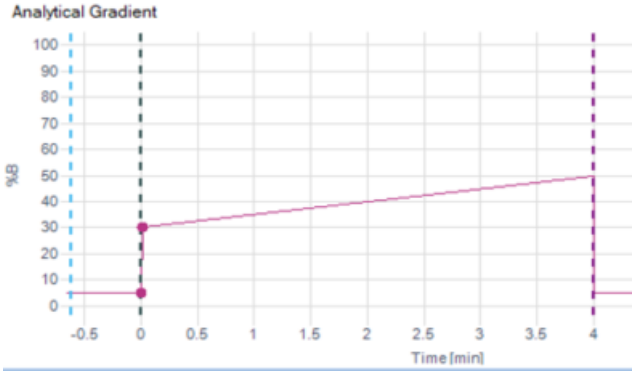
Figure 115: Preview of the display of the analytical ^{2}D gradient in purple and the flush in orange

NOTE If the ^{2}D gradient view is activated in the main window, the Y-axis shows %B.

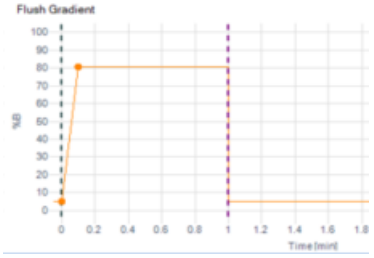
This tool toggles the display of the threshold and slope values at the cursor position in the Preview. This tool can only be used if the gradient preview in the main panel is deactivated. For manually changing the threshold setting in the preview, see below



Using this tool will toggle the display of the ^{2}D analytical gradient panel at the right of the Preview.



To switch between the analytical gradient and the flush gradient in the right panel, click this tool.



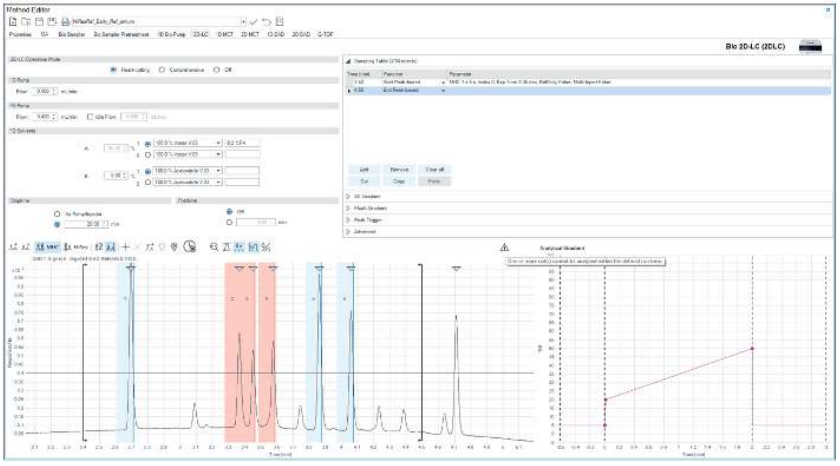
The tool is unavailable when the analytical gradient is used as a flush gradient.

Further Graphical Explanation

Further Graphical Explanation of the 2D-LC Preview Window:

The grey triangle illustrates which peaks the peak trigger settings detect in the reference chromatogram. To add or remove the cut, double-click the grey triangles in the preview.

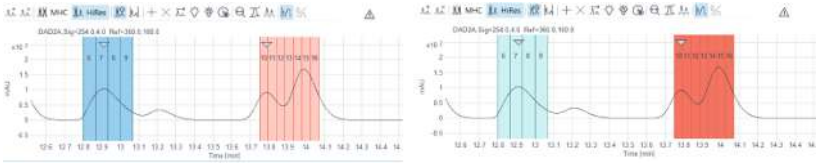
The grey line in the preview marks the stop time.
In the following example, the **Stoptime** is too short to analyze all cuts. Therefore you must change the stop time to 57 min as indicated by ^{2}D-gradients. For example use the optimization tool or double click the grey line (green arrow) marking the current stop time. Then the SW automatically adjusts and takes up the stop time. The alert icon will disappear (unless anything else is wrong).



NOTE: Hovering over the alert icon gives an idea of what's wrong.

Marked cuts

- **Marked cuts** are displayed either in dark blue bars (can be analyzed) or in dark red bars (cannot be analyzed).



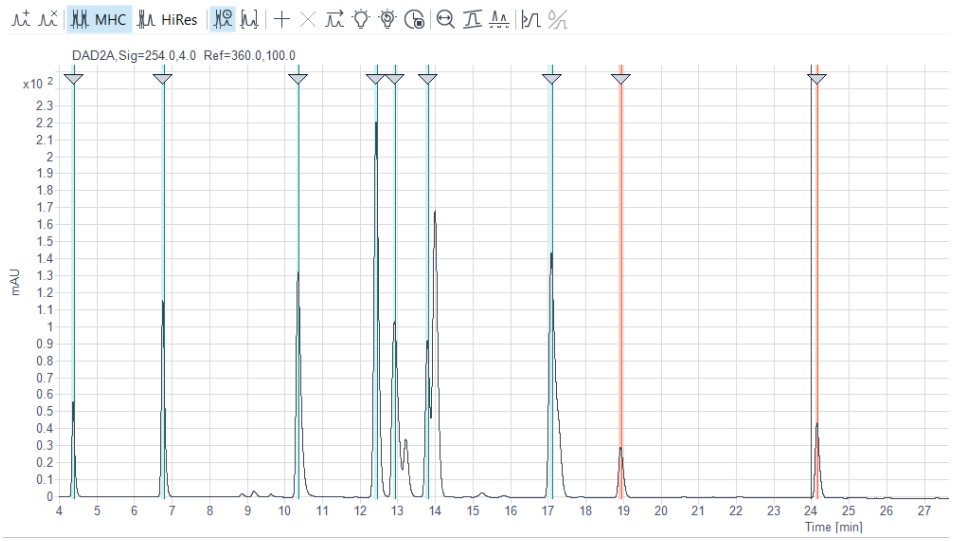
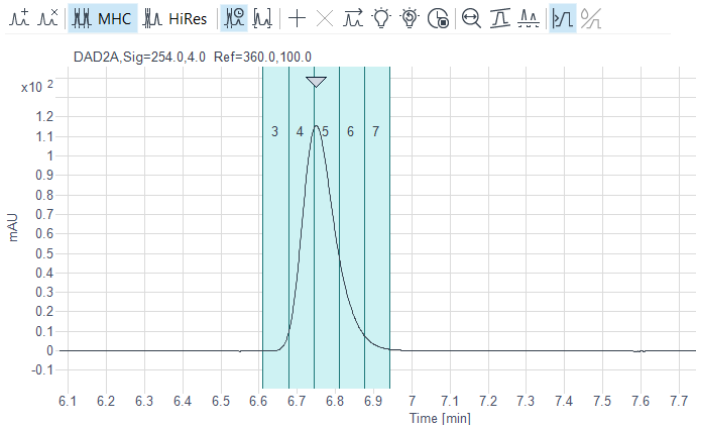
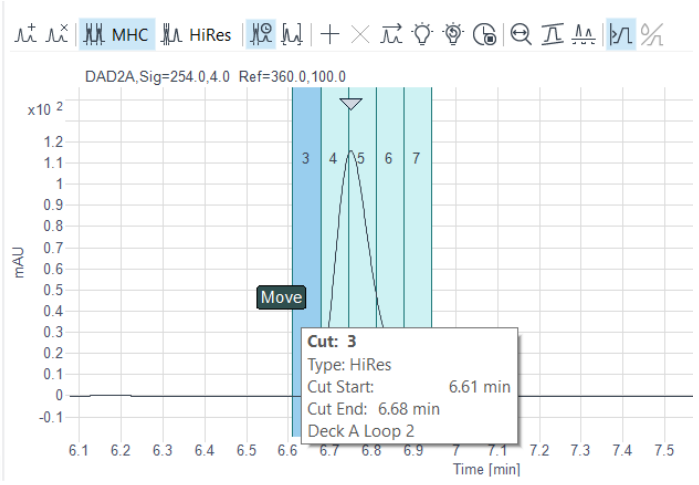
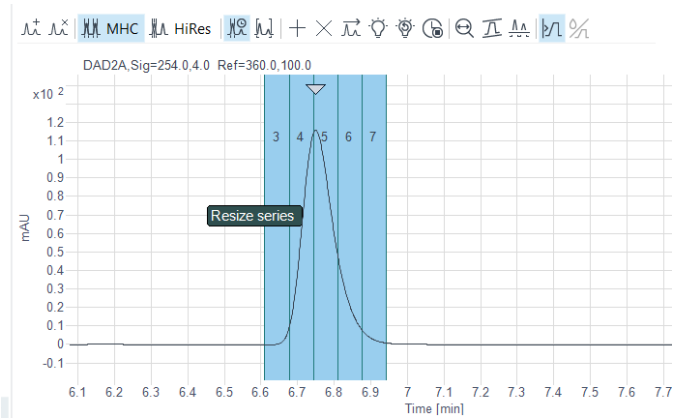
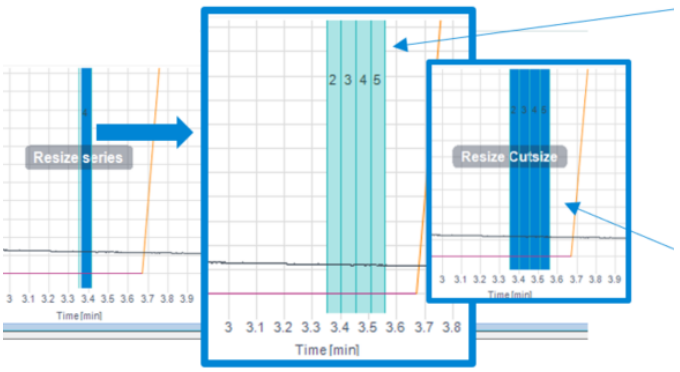
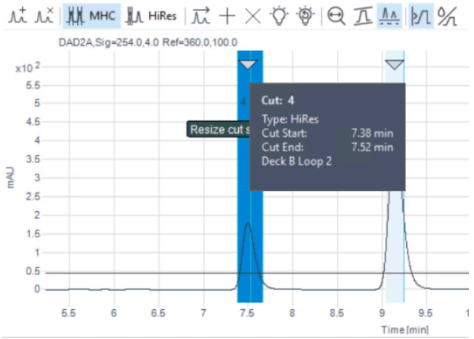
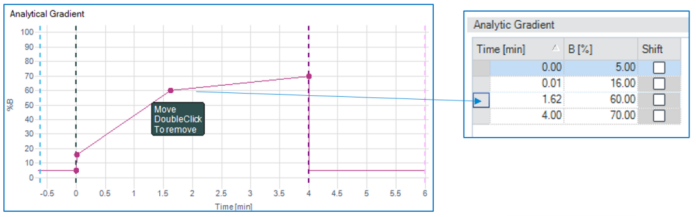


Figure 116: Chromatogram with missed peaks marked red

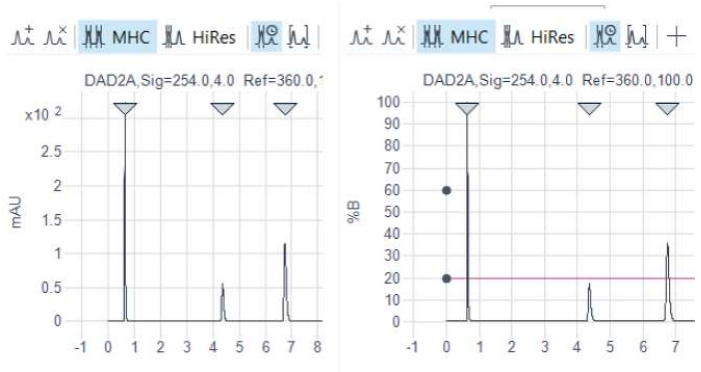
MHC cuts (time based)	This function uses the continuous flow-through principle. The cuts are visualized as light green bars. The dark line on the right edge of the bar indicates the switching time of the 2D-LC valve and the end of parking the peak. Cuts can be marked and moved to another position in the preview window.
HiRes cuts (time-based)	**HiRes cuts (time-based)** are visualized and marked as light green bars. Depending of the peak width the cuts can vary from 2 to maximal 10 cuts. Compared to MHC, HiRes cuts have two dark lines one on the left side one of the ride side of the bar which reflects the switching before and after parking a peak. The left dark line defines the start time of one High-Resolution Sampling event. 

MHC cuts / HiRes cuts (peak-based)	**MHC cuts / HiRes cuts (peak-based)** are displayed graphically in blue bars. Hovering over the bars gives you the option to move the HiRes Sampling.
Move cut	 You can: • Increase or decrease the cut size • Grab the highlighted cut and move to another time The sampling table takes up the new times

Resize the HiRes series	Hovering over the bars gives you the option to increase or decrease the cut series (indicated by green highlights). You can resize by: • Clicking the highlighted series and dragging the edge along  • Dragging one of the inner edges  **NOTE:** For **HiRes**, these changes of cut size and number of cuts can also be made in the sampling table. **NOTE:** For **HiRes**, these changes of cut size and number of cuts can also be made in the sampling table.For **HiRes**, even if several cuts are programmed, this does not mean that all cuts are performed. If the threshold is reached a second time, sampling is aborted.

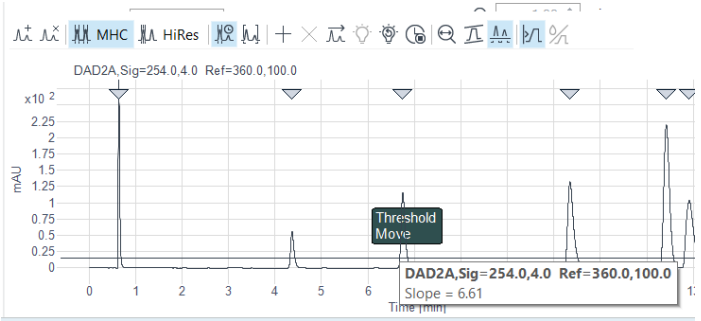
Cut Information	Hovering over highlight bars give you more cut information, like cut number, start and end time of the cut and in which deck and loop the cut is parked. Also ^{2}D gradient / i.e. time of analysis is indicated. 
Setup ^{2}D gradient graphically	The initial ^{2}D gradient in the **Analytical Gradient** preview by double click purple line adds a purple ball, which can be moved around to change the initial gradient. Analysis and equilibration time can be adjusted in the Preview by moving around corresponding lines. The tables take this up.  **NOTE:** To add another gradient point, double click the purple line.

Activate the ^{2}D gradient view will display the name of the Y-axis in %B.



Threshold

If the threshold is activated, you can grab this line and shift up and down to adjust the threshold.
This measure will also update **Peak Trigger** settings.
If you hover over the threshold line, the slope is also displayed at the intersection with the peak signal.



Set up a Peak-Based Experiment Graphically

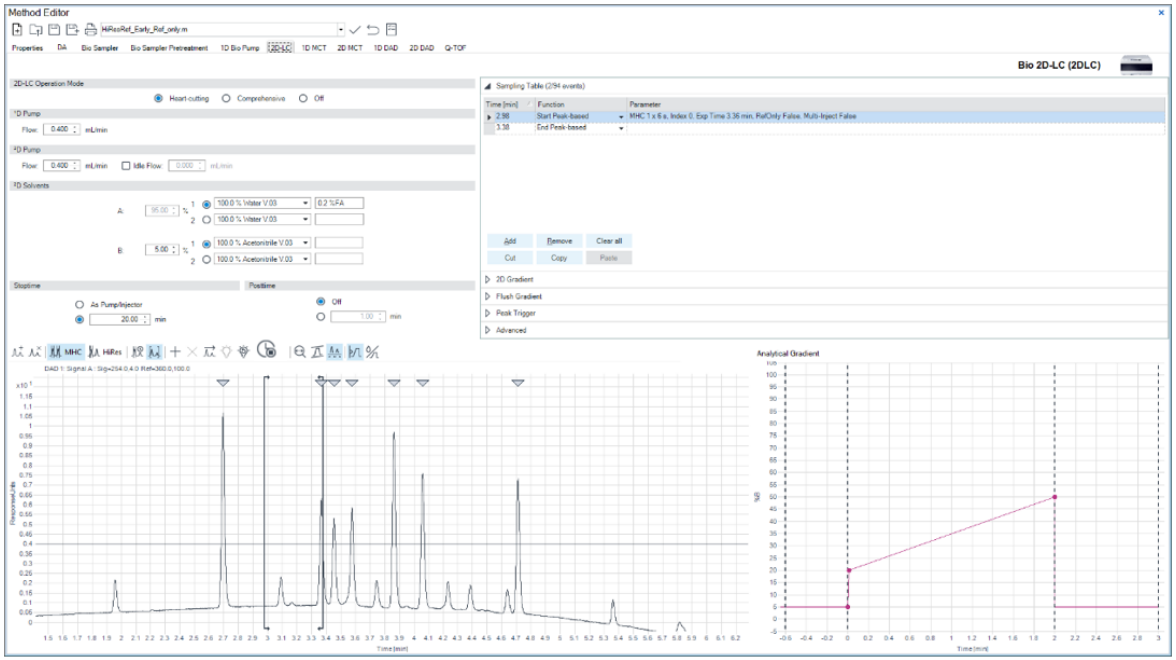


Figure 117: Peak-based experiment based on a prediction

In peak-based mode, the [1]D detector triggers sampling/parking of cuts in dependence on a UV-threshold (or slope) A peak appears in the detector, the threshold is reached (= peak start), the 2D-LC modulator starts sampling. A sampling time can be defined, which determines the max. sampling time for peak-based cuts. If peak-end is detected before the sampling time has finished, sampling is stopped. The event that comes first will define the time for peak-based sampling. Adjust the peak-based area either by grabbing start and end bracket and moving along in the preview or by adjusting the times in the sampling table.

1 Upload chromatogram into preview.

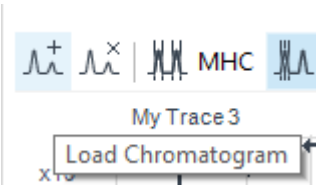


Figure 118: Load Chromatogram

2 Define UV-threshold and mark threshold symbol for display.

3 Select Detector and Signal used for triggering.

In this example G7117A and Signal A are selected.

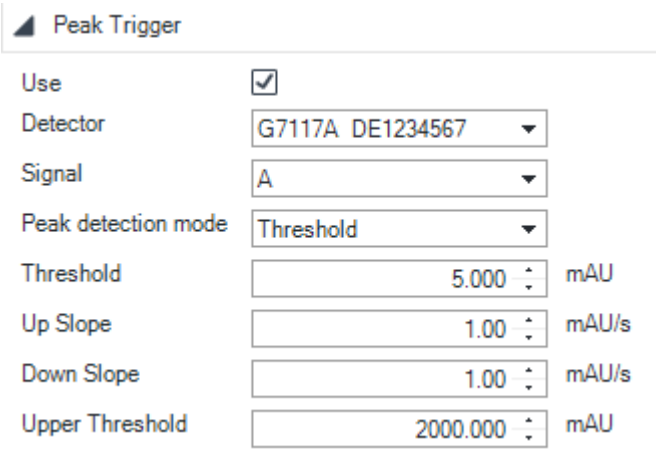


Figure 119: Peak Trigger view

4 Select MHC or HiRes.

The icon corresponds to the peak-based operation. In this example **MHC** is selected.

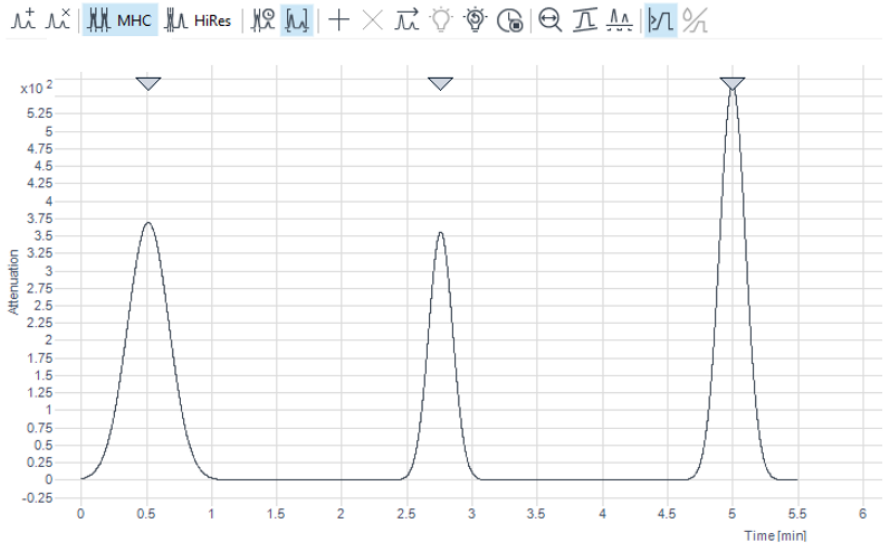


Figure 120: MHC chromatogram

5 Double-click one of the grey triangles located above the chromatogram.

A bracket appears, which marks a peak-based area (start and end peak-based) which is taken up in the sampling table.

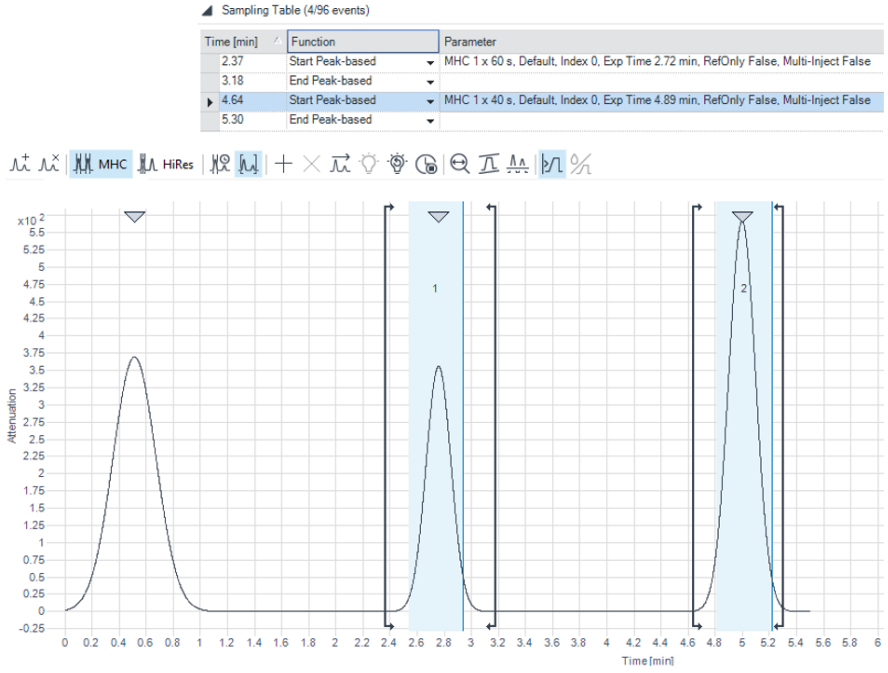


Figure 121: Two selected peaks in peak based mode and corresponding peak-based sampling table

NOTE

To generate a peak based event in the preview, you can also use the add icon or do a right click somewhere into the preview, and press **add cut**.
In the sampling table you can add the events start and end peak-based, see Use Peak Trigger on page 178 .
For adjusting the peak-based area it can be either be done by grabbing start and end bracket and moving along in the preview or by adjusting the times in the sampling table.

6 If some highlights appear in red hovering over the warning triangle tells you that stop time must be adjusted. To adjust the stop time the stoptime button must be clicked pressed. Then the Stop time has been prolonged to ensure that all predicted cuts can be ^{2}D analyzed.

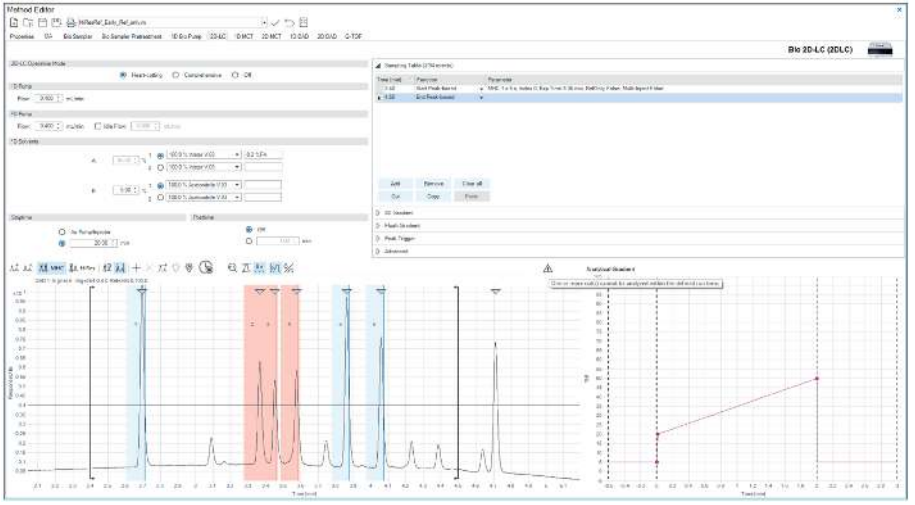


Figure 122: Cut 3, 4 and 5 are shown in red. The warning triangle one or more cut(s) cannot be analyzed within the defined run time

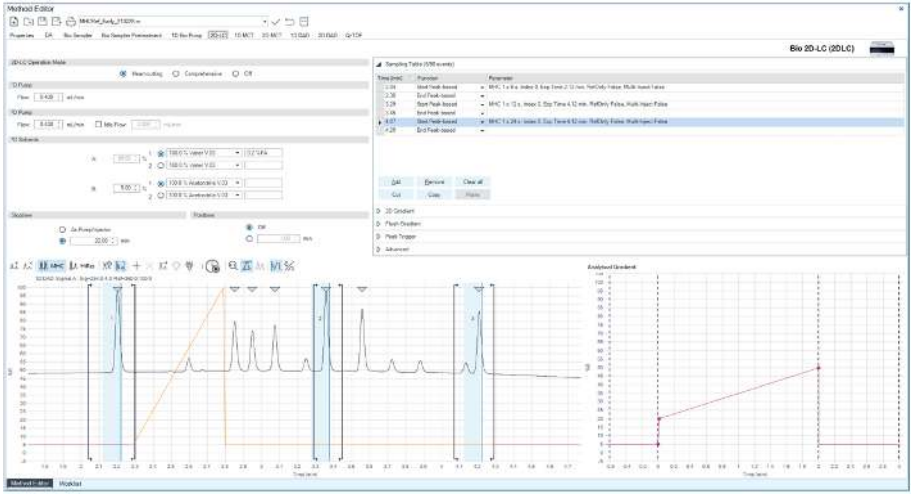


Figure 123: Example Uploaded chromatogram with 3 peak based areas in MHC mode

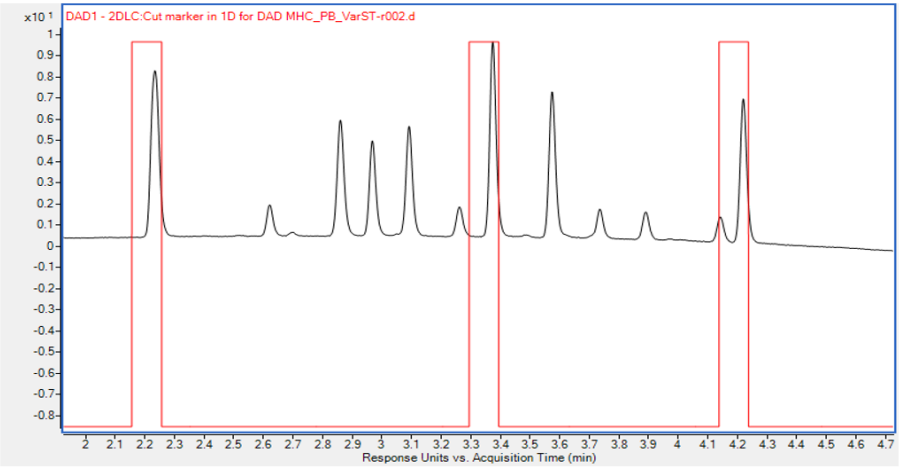


Figure 124: Example shows result of displayed in the MassHunter Qual 1D-Chromatogram + Cut markers

NOTE

This example is based on a prediction. For experiments with unknown outcome you have to add an extra time to the stop time for cases where you don't know what to expect.

Setup ^{2}D Gradient Graphically

1 Load the initial ^{2}D gradient in the Analytical Gradient Preview.

2 To change the initial gradient, double click the purple line.

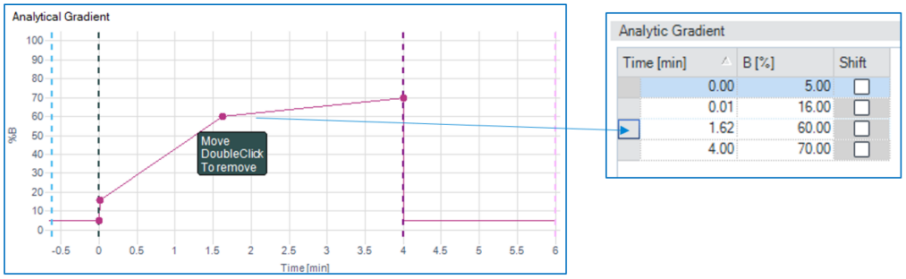


Figure 125: Analytical gradient

This adds a gradient point to the line (purple point). This gradient point can be moved. To add another gradient point, double click the purple line again.

To adjust analysis and equilibration time, move the corresponding lines. All is taken up by tables.

3 To display the name of the y-axis in %B, activate the ^{2}D gradient view.

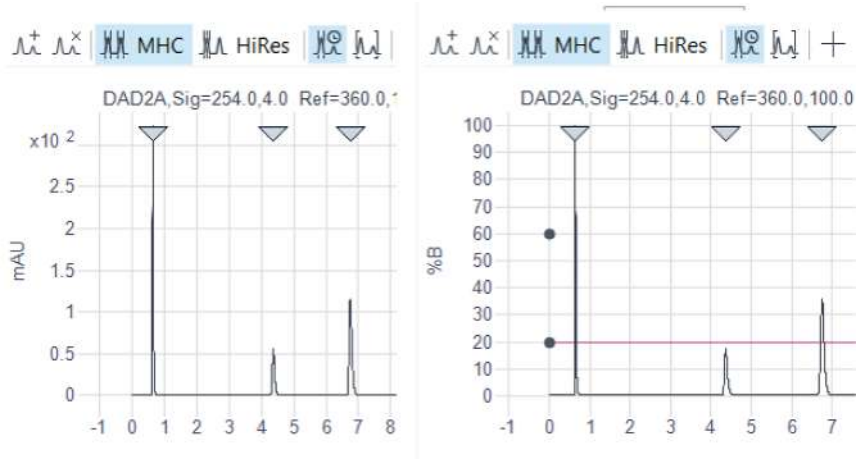


Figure 126: Changing the Labeling of the coordinate axes

4 Click .

This activates the threshold line. To adjust the threshold, grab this line and shift up or down. This will update the peak trigger settings. If you hover over the threshold line, the slope is also displayed at the intersection with the peak signal.

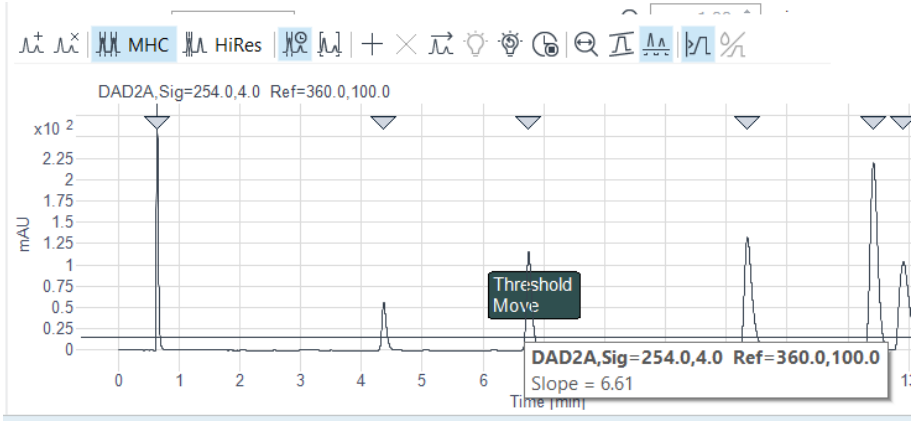
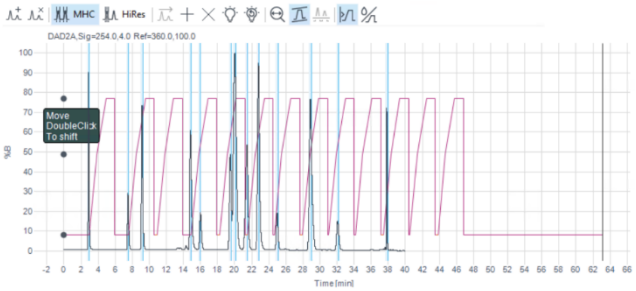
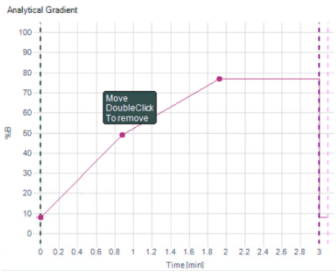
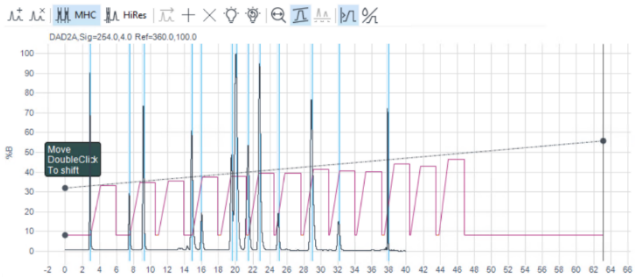
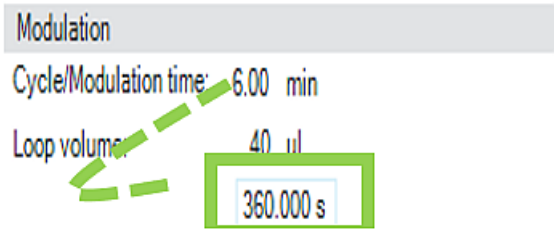
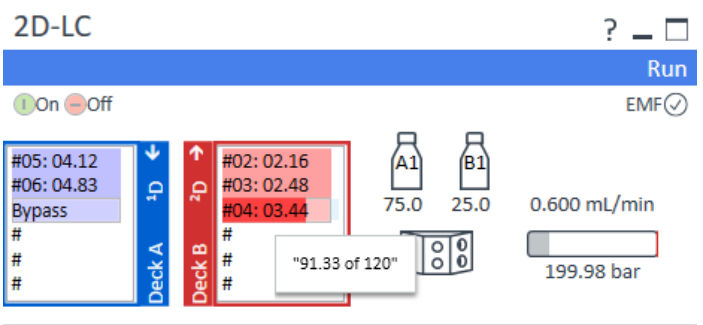


Figure 127: Display the threshold in the preview

Set up Second Dimension Gradient with the Graphical User Interface

The user can graphically set up the ^{2}D gradient including the initial composition (%B) value, the ^{2}D stop time, and the modulation (repetition) time.

Analytical Gradient	You can change or adjust the values of the **Analytical Gradient** graphically. In the preview, select one of the black bullets with the mouse and move the bullet up and down. These changes will automatically update the **Analytical Gradient** settings in the table. By double clicking on the line in the **Analytical Gradient** window, you can set more anchor points to adjust the analytical gradient even better.  
Gradient Shift ¹D Time (Shifted ²D gradient)	The setup of the shifted gradient can also be done graphically. If you double click one of the black bullets in the preview window, you will get a dotted line, which represents the shifted ²D gradient. By moving the bullet up and down, you can align the shifted ²D gradient to the different solvent composition from the ¹D run. By double clicking on the dotted line again, you can set more anchor points to adjust the shifted gradient even better. These changes will automatically update the **Gradient Shift ¹D Time** settings in the table.  **NOTE:** Within a **HiRes** series shifted gradients are prohibited, but shifts are allowed from **HiRes** series to series.

More Modulation Information	Hovering over cycle time and or time for ASM flush-out displays actual time in second: Three digits (for comprehensive mode). 
More Info 2D-LC Valve Online monitoring	Hovering over analysis loop indicates time passed and time remaining (in seconds) More info about online, see 2D-LC Valves Online Monitor in the 2D-LC User Interface on page 141. 

Additional Information

Multi-Inject

To sample a broad 1D-region that does not fit into currently installed sampling loops (e.g. 40 µL volume) HiRes is the method of choice. Take in account, that this leads to an increased number of ^{2}D cycles. Multi-Inject allows to define a HiRes group to being injected at once, which means the content of the loops is transferred to the ^{2}D column before a single ^{2}D gradient is used for analysis.

NOTE Maximum number of a HiRes cut group (i.e. the number of cuts that can be made with one HiRes entry in the time table) is 10 at most, regardless of whether Multi-Inject. If a deck is free again, 5 HiRes cuts of a new group can be parked there again with certainty, everything else depends on the further timing.

1 To inject a HiRes group at once, select Multi-Inject.

The content of the loops is transferred to the ^{2}D column before a single ^{2}D gradient is used for analysis.

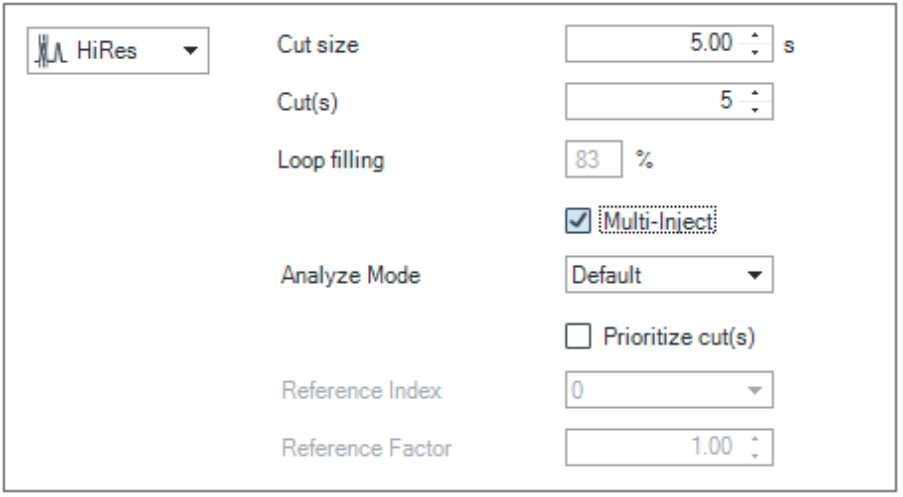


Figure 128: Multi Inject for High-Resolution Sampling

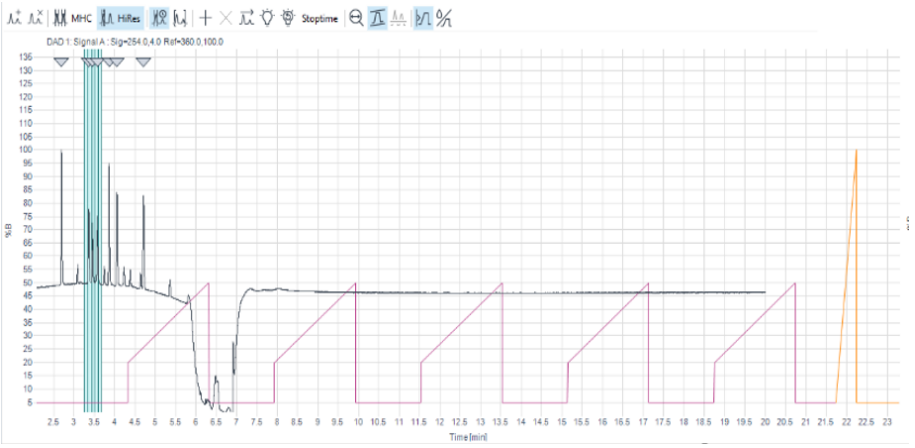


Figure 129: Example of High-Resolution Sampling (5 cuts) with 5 analytical gradients

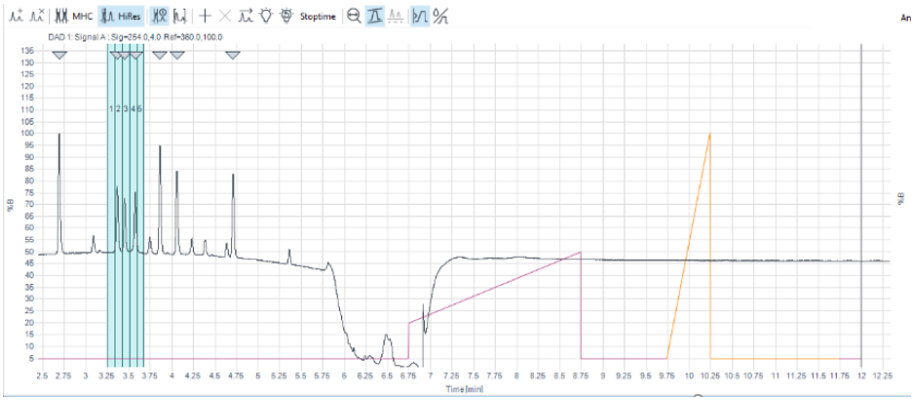


Figure 130: Example of High-Resolution Sampling (5 cuts) with only one analytical gradient for all cuts

NOTE

Multi-Inject works similar to an injection from a large sample loop. Large injection volumes can negatively affect ^{2}D separation. Consider a good ^{2}D retention by starting at low percentages of B and by applying ASM. Therefore, Multi-Inject is not recommend for volume-based isocratic separations, e.g. SEC.

Dynamic Peak Parking

In certain cases, small variations of parameters can influence changes in the retention time (RT) mechanism. This can happen, for example, with certain types of analytes such as peptides. As a solution to compensate for such effects in Time-based (M)HC 2D-LC experiments, the Dynamic Peak Parking is used. “Dynamic Peak Parking” uses an internal RT-standard (IRTS), which is detected by using peak-based mode. If the “expected time” of this IRTS shifts to earlier or later, subsequent time-based cuts linked to the IRTS will be adjusted accordingly.

Setup an IRTS experiment for heart cutting mode

1 Upload the chromatogram into the preview.

2 Define the UV-threshold (peak trigger) such that the expected IRTS is predicted to being sampled, see *How to setup the peak-based experiment*.

3 Define the peak-based area (start and end peak -based) in which the IRTS is expected.

This can be done for instant via Sampling table or by selecting the icons peak based plus/ MHC or HiRes in the preview UI and double clicking on the triangle of the peak of interest (IRTS), see *How to setup the peak-based experiment*.

4 If needed, adjust the peak-based area either by grabbing start and end bracket and moving along in the preview or by adjusting the times in the sampling table.

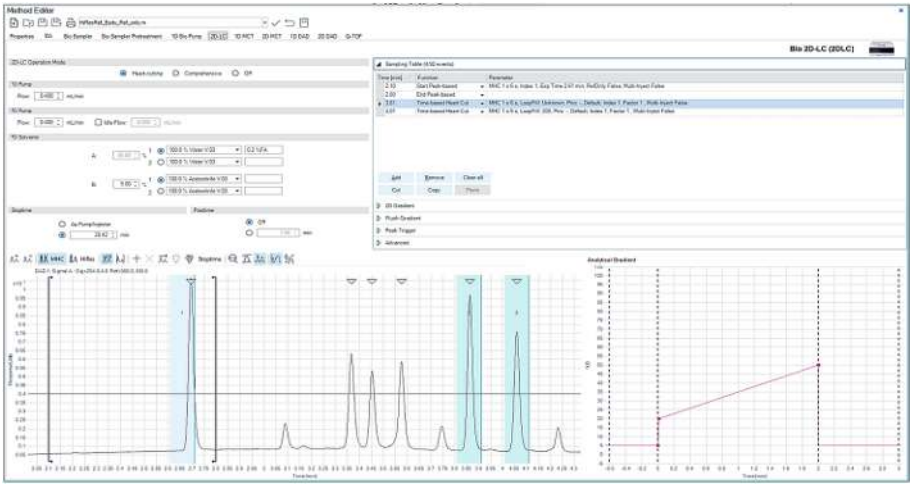


Figure 131: Peak-based heart cutting experiment

5 Verify in the sampling table the expected time for the IRTS, which corresponds to the peak-start trigger in the preview (intersection of threshold line and peak front). Then define the Reference Index value for IRTS, which is then shown in sampling table. For the first IRTS like in this example the value is 1.

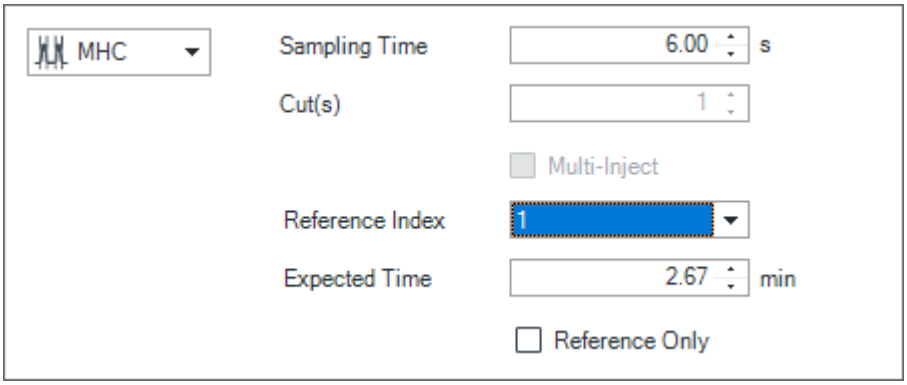


Figure 132: Parameters of the IRTS defined as peak based MHC

NOTE The IRTS will be 2D-analyzed unless you mark the field Reference Only. Then the IRTS is detected, the time shift applied to all following time-based cuts but the IRTS will not be analyzed.

NOTE In case you change your threshold after having defined the IRTS you need to update the expected time, which is done by double clicking the peak-based start bracket.

NOTE The first peak in the defined area is always used as Reference Peak and if **Reference Only** is selected the peaks are not parked no matter how many are in the range.

6 If a Reference Index has been defined for the IRTS, then all following time-based cuts automatically get the Reference Index value and are so linked to IRTS with this index.

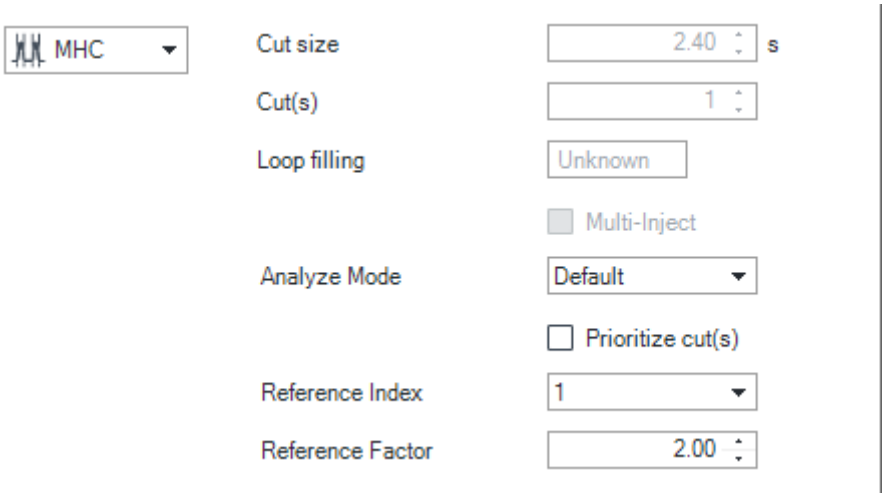


Figure 133: Time based cut with Reference Index 1 and Reference Factor 2

The standard value 1 for the Reference Factor will work for simple linear shifts. To determine the Reference Factor more precisely, it should be determined experimentally (e.g. a shift by 1 min with a factor of 2 would shift the time-based peaks by 2 min).

Example of a Dynamic Peak Parking Setup

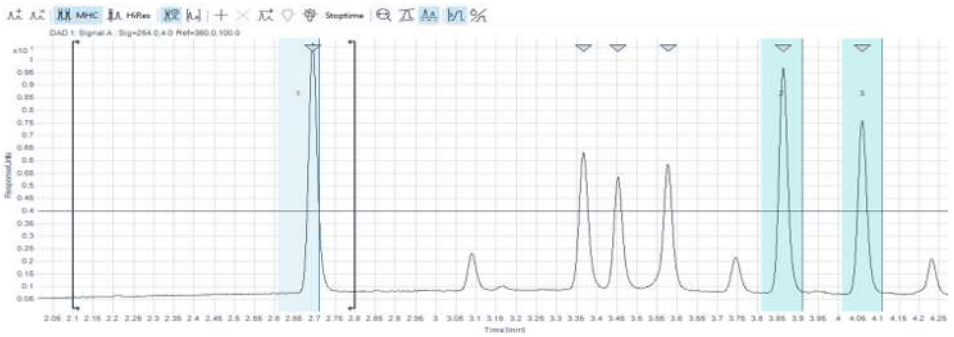


Figure 134: Example picture of the dynamic cut shift setup

Table 21: IRTS

Peak-based start:	2.10 min
Peak-based end:	2.80 min
Expected time:	2.67 min

The maximum shift of the IRTS is 0.57 min. This means the end of peak-based area is at 2.80 min and the next time-based cut with reference to IRTS can be placed at 2.8 + 0.57 = 3.37 min.

NOTE

If a time-based cut shifts to the front and would enter the peak-based area (bracket) the dynamic cut shift will not work.

Here is an example of how fluctuations that occur during a run can be compensated with the help of the IRTS and dynamic cut shifting.

The chromatograms below shows the results obtained from MassHunter Qual. The top 1D chromatogram shows the original reference chromatogram the method was based on. The middle 1D chromatogram indicates the RT shift.

The cut-markers image below show that time-based cuts were dynamically shifted accordingly.

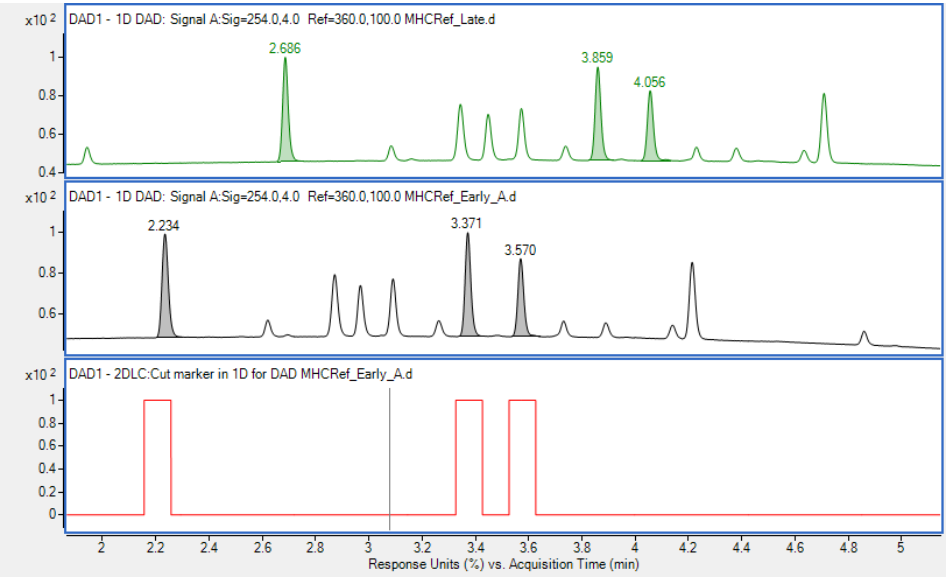


Figure 135: Shift of IRTS (2.686 min) to earlier RT (2.234 min) which is compensated by the system

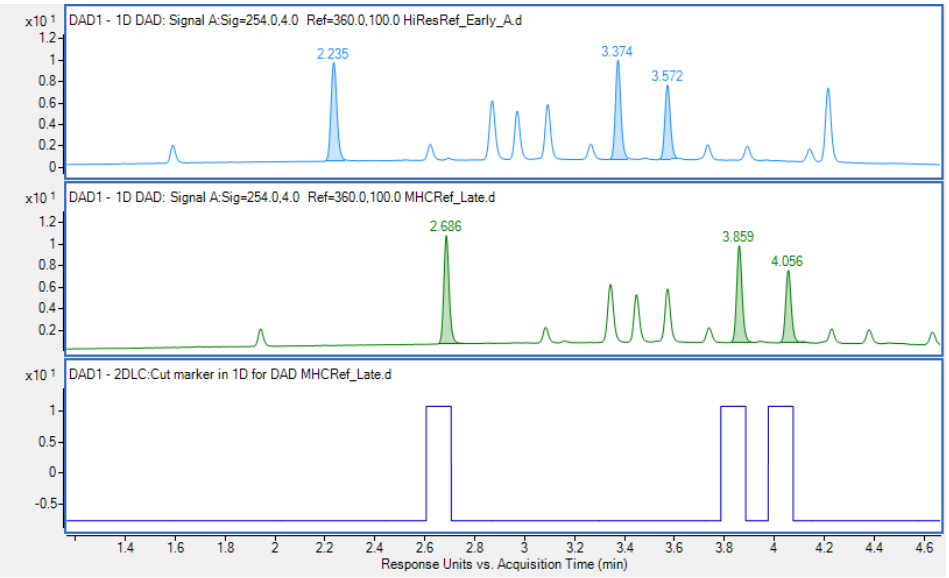


Figure 136: Shift of IRTS (2.235 min) to later RT (2.686 min) which is compensated by the system

Modulation Information	Hovering over Cycle time and or time for ASM flush-out displays actual time in second: 3 digits (for comprehensive mode)

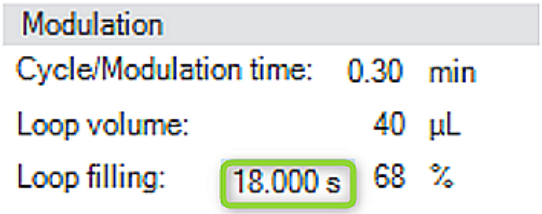


Figure 137: Modulation

NOTE

To avoid rounding errors in the transfer of the exact modulation time (LCxLC) to third party data analysis system, use three decimal places.

2D-LC Valve Online monitoring	Hovering over analysis loop indicates time passed and time remaining (in seconds).

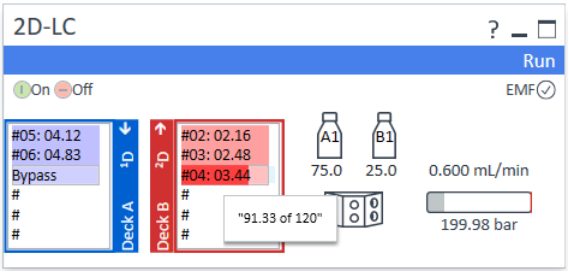


Figure 138: 2D-LC Online Monitor in the user interface

7 Method Development of Active Solvent Modulation (ASM)

This chapter provides information on how to develop methods when using Active Solvent Modulation (ASM).

Method Development of Active Solvent Modulation (ASM)

ASM method development helps finding the optimal dilution of 1D solvents in the sample loop for best 2D resolution at lowest cycle time.

After switching on the ASM functionality (see Method Parameters on page 223), execute the steps in the following order:

- Optimize the Dilution by Using ASM Capillaries on page 224
- Optimize the Sample Loop Flush on page 225
- Include the ASM Phase to the ^{2}D Gradient on page 226
- Optimize Dilution Through Method Settings on page 227

Method Parameters

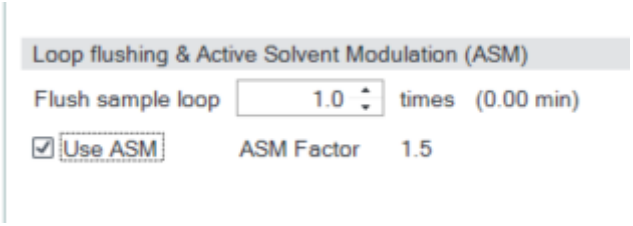


Figure 139: Loop flushing and Active Solvent Modulation (ASM)

Advanced settings of 2D-LC method parameters allow switching on and off the use of the ASM functionality.

- If this option is off, it works as a standard 2D-LC valve without dilution.
- If this option is on, the user can set how often he wants to flush the sample loop during the ASM phase.

Optimize the Dilution by Using ASM Capillaries

A choice of four different ASM capillaries is available for achieving best results. Longer capillaries reduce, shorter capillaries increase the dilution of 1D solvent in the sample loop. Install and configure different ASM capillaries, see Connecting the 2D-LC Valve, ASM (G4243A) on page 65 for optimizing the results.

Table 22: Available ASM Capillaries and properties

Capillary p/n	Length (mm)	Inner diameter (mm)	Volume (µl)	ASM factor	Split ratio (loop:ASM)
5500-1300	85	0.12	0.96	5	1:4
5500-1301	170	0.12	1.9	3	1:2
5500-1302	340	0.12	3.8	2	1:1
5500-1303	680	0.12	7.7	1.5	1:0.5

Optimize the Sample Loop Flush

Activate ASM in the software and set Flush sample loop to 3.0 times.

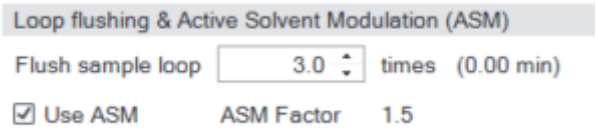


Figure 140: Loop flushing and Active Solvent Modulation (ASM)

NOTE

Flushing the sample loop 3 times is typically enough and the recommended default. Less time may be sufficient and can be verified during optimization. The user interface displays how long this will take.

Include the ASM Phase to the ^{2}D Gradient

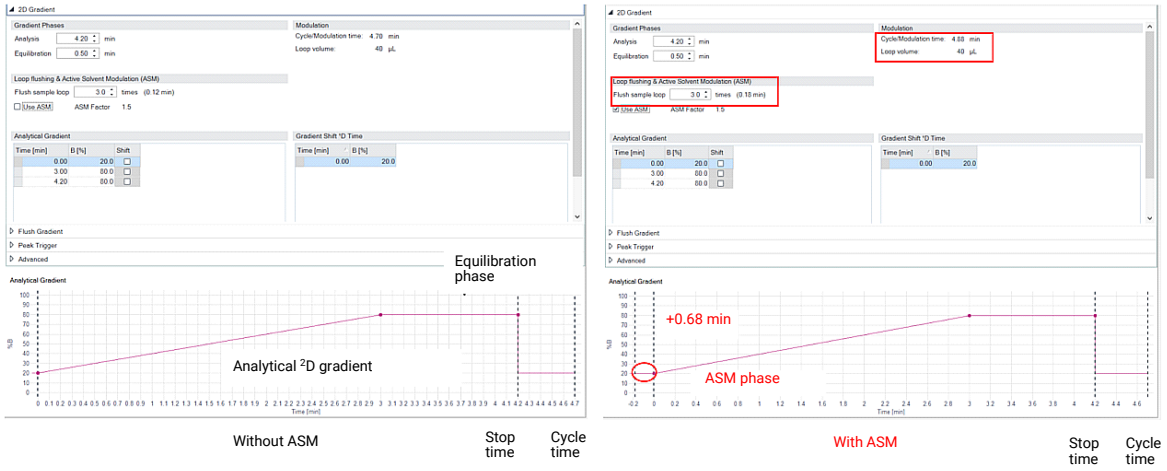


Figure 141: Programming the ^{2}D gradient table (example)

The dilution during the ASM phase takes time. That's why the ASM phase shifts the analytical gradient start.

An ASM phase of, for example, 0.68 min (based on selected ASM capillary, flush factor and ^{2}D flow rate) shifts all times by 0.68 min compared to a ^{2}D gradient without ASM

- Gradient ends later
- ^{2}D cycle time is increased accordingly
- **Use ASM** automatically added an ASM phase
- Before the actual gradient and ASM phase takes place, done by the software

This rule is true for shifted gradient steps as well (if applicable).

Optimize Dilution Through Method Settings

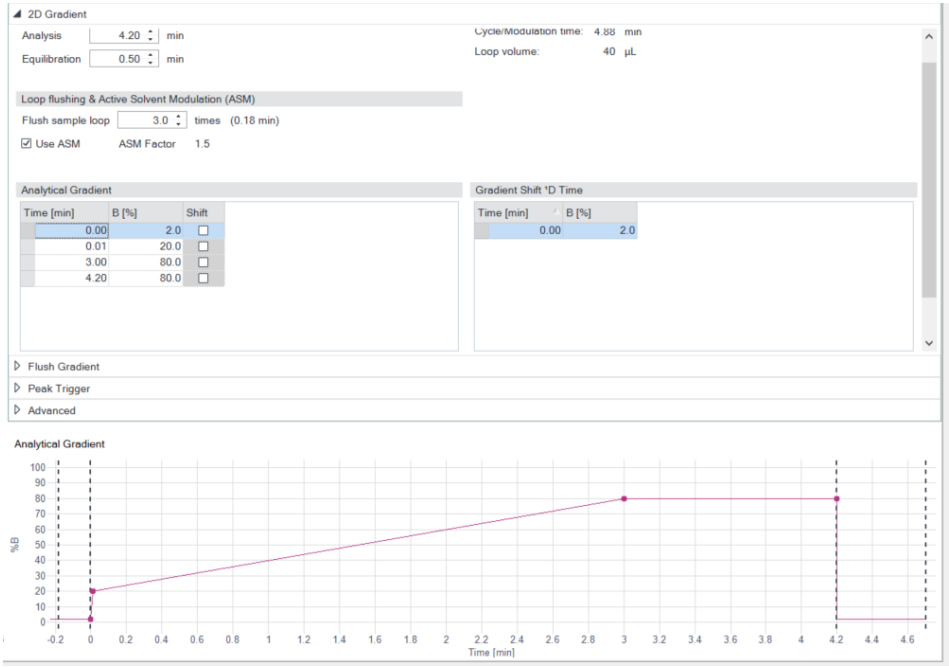


Figure 142: Optimizing separation using a lower percentage of B for the ASM and column equilibration phase (example)

For optimizing separation, you may use a lower percentage of B for the ASM phase and column equilibration phase compared to the original gradient for increasing dilution before the ^{2}D column.

If for example the original analytical gradient started at 20 % B, you may use an ASM phase of for example 2 % B for diluting ^{1}D solvent more strongly during the ASM phase by changing the gradient start condition and adding a line to the 2D gradient table for the ASM phase. The starting point for the analytical gradient does not change. The solvent composition of the equilibration phase is automatically reduced to the start condition.

Apply High-Resolution Sampling with small cut sizes. Small cut sizes reduce the transfer of solvent volume from ^{1}D to ^{2}D, which can further improve solvent compatibility and 2D resolution.

8 Run the System

This chapter describes how to run the 2D-LC Solution in the modes standard heart-cutting, multiple heart-cutting, high-resolution sampling and comprehensive 2D-LC with the driver-based 2D-LC Solution.

Introduction to Start a System Run

The introduction procedure illustrates the system´s 2D-LC capabilities and supports the user to start the method for a specific analytical task. The introduction procedure will guide the user through the most important setups and analysis function.

The sample provided with the introduction procedure can be determined with a UV-detector and a mass spectrometer. The methods to analyze the starter sample are delivered together with the full package to ensure a smooth introduction and checkout procedure. With the given method, peaks will overlap in the first dimension and will be separated in the second dimension.

The2D-LC Solution is delivered together with all required parts for a complete introduction procedure for (multiple) heart-cutting and comprehensive 2D-LC.

NOTE

Methods for system preparation and checkout runs are available on the Agilent 2D-LC Software data media for recommended system configurations. Not all possible configurations can be shown here. Therefore, adapt these methods for other configurations and modules if necessary.

Prepare the 2D-LC System

Prepare the 2D-LC System for LC

As a user guide for good preparation, refer to the help instruction and suggestions of Good Laboratory Practice for HPLC.

1 Condition your Agilent HPLC instrument to have a stable system.

2 For further details, see *Best Practices for Using an Agilent LC System Technical Note (InfinityLab-BestPractice-en-SD-29000194.pdf, SD-29000194)*, or the user manual of each module.

Prepare the 2D-LC System for MS

The ion source parameters depend on the composition and flow rate of the mobile phase being used. Therefore it is usually worth retuning the mass spectrometer, after the LC conditions have been determined.

1 Perform an Auto Tune.

2 To clean the source and flush the LC/fluidics lines out prior to starting your experiment, use LC-MS grade solvents and reagents.

This measure ensures that the optimum sensitivity is achieved, improves reproducibility, and avoids many common problems.

3 Check additional parameters like the Source Temperature Drying Gas Temperature and Gas Flow.

Such parameters are rarely adjustable during the analysis and should be optimized before starting the analysis.

4 For further LC-MS details, refer to the documents such as the user manuals, user guides and instructions for the respective modules.

Configure the 2D-LC System

The introduction refers to the driver-based 2D-LC solution. The 2D-LC software requires at least the following CDS versions:

Preparations

- MassHunter Workstation 11 for QTOF/TOF (or higher)
- MassHunter Workstation 12 for TQ (or higher)
- For further details like firmware and driver, see MassHunter Workstation Data Acquisition on page 46.

Configure 2D-LC Hardware

Focus on the 2D-LC Valves and the capillary connection.

1 To find out the correct plumbing of the 2D-LC valve ports, see Connecting the 2D-LC Valve, Standard (G4236A) on page 62, Connecting the 2D-LC Valve, ASM (G4243A) on page 65, or the 2D-LC online help.

The recommended plumbing of the 2D-LC valve differs between 2D-LC setup with single loops versus 2D-LC setup with Multi Heart Cutting (MHC) Valves and concurrent versus countercurrent mode.

Table 23: Hardware setups for 2D-LC modes

2D-LC mode	Hardware setup
Standard heart-cutting	• 2D-LC Valve with one single loop • 2D-LC Valve with two single loops • 2D-LC Valve with two MHC valves (each with six Sample Loops) • 2D-LC ASM Valve with two MHC valves (each with six Sample Loops)
MHC or HiRes	• 2D-LC Valve with two MHC valves (each with six Sample Loops) • 2D-LC ASM Valve with two MHC valves (each with six Sample Loops)
Comprehensive	• 2D-LC Valve with two single loops and 2D-LC Valve with two MHC valves (each with six Sample Loops) • 2D-LC ASM Valve with two MHC valves (each with six Sample Loops)

NOTE 40 µL sample loops are part of the default setup in the methods of the data media.

NOTE The 2D-LC valve with one single loop setup is only used for special applications, for example the Bio ProtA-Sec Kit.
For more information, see the bio application documentation.

NOTE Methods for preparation and checkout runs of recommended system configurations are available on the Agilent 2D-LC Software data media.
Not all possible configurations can be shown here. Therefore, adapt these methods for other configurations and modules if necessary.

Configure 2D-LC Software

1 Configure the 2D-LC solution as **2D-LC Cluster**, see Configure the 2D-LC Cluster on page 119.

2 To check the correct selection of the individual components like sample loop, transfer capillary and ASM capillary (if applicable), use the context menu function **Modify**.

Correct the selection if necessary.

3 Load the given reference method.

NOTE If you want to load and use a 1D method instead of a 2D method, make sure that the 2D-LC mode is deactivated.

NOTE System preparation and checkout run methods for recommended system configurations are available on the Agilent 2D-LC Software data media. Other configurations and modules require an adaption of the methods.

4 Check modes (Heart cutting or Comprehensive) and all other important parameters in the method before starting the run.

NOTE Except the pumps, all other units should have the pump set as the stop time.

Checkout Procedure

The checkout procedure requires 5190-6895 (2D-LC starter sample, 1 x 2 mL) , that contains the following components.

Table 24: Components of 5190-6895

Analyte	CAS#
Atrazine	001912-24-9
Atrazine-desethyl	006190-65-4
Chlorotoluron	015545-48-9
Diuron	000330-54-1
Hexazinone	051235-04-2
Linuron	000330-55-2
Metazachlor	067129-08-2
Methabenzthiazuron	018691-97-9
Metobromuron	003060-89-7
Metoxuron	019937-59-8
Nifedipine	021829-25-4
Nimodipine	066085-59-4
Prometryn	007287-19-6
Sebuthylazine	007286-69-3
Terbuthylazine	005915-41-3
Terbuthylazine-desethyl	030125-63-4

The method parameters described here have been optimized for the following hardware configuration.

Table 25: Hardware configuration for optimized method parameters

	^{1}D	2D-LC	^{2}D
LC	ALS	Universal drives with 2D-LC ASM valve and two MHC valves	
	Pump		Pump

	^{1}D	2D-LC	^{2}D
	MCT		MCT
	UV Detector		UV Detector
LC-MS			High-End mass spectrometers

Prepare the Experiment

Parts required

Qty.	p/n	Description
1	5190-6895	2D-LC starter sample, 1 x 2 mL
1	G2453-85060	Formic Acid-Reagent Grade 5 mL (5 cc)
1	685775-902	InfinityLab Poroshell 120 SB-C18, 2.1 x 100 mm, 2.7 µm In ^{1}D for ESZ Service
1	699968-301	Poroshell 120 Bonus-RP, 3.0 x 50 mm, 2.7 µm In ^{2}D for ESZ Service

Various hardware configurations are possible, see Options on page 54.

Take care that the following solvents for mobile phases are available:

Preparations

1D

- A = water with G2453-85060 (Formic Acid-Reagent Grade 5 mL (5 cc))
- B = methanol

Preparations

2D

- A = water with G2453-85060 (Formic Acid-Reagent Grade 5 mL (5 cc))
- B = acetonitrile

NOTE Recommended to use legacy setup for the old columns and easy start kit for the new columns.

Preparation of 1.2 mL sample (1:10) for standard LC

1 To prepare 1080 µL dilution solvent, add 216 µL methanol to 864 µL Mobile Phase A. 1080 µL dilution solvent (20 % methanol in mobile phase A) is prepared.

OR: To prepare 3600 µL dilution solvent, add 720 µL methanol to 2880 µL Mobile Phase A. 3600 µL dilution solvent (20 % methanol in mobile phase A) is prepared.

2 To prepare 1.2 mL sample (1:10), add 120 µL 2D-LC starter sample to 1080 µL dilution solvent.

OR: To prepare 4.0 mL sample (1:10), add 400 µL 2D-LC starter sample to 3600 µL dilution solvent.

Dilution of the 2D-LC starter sample in a ratio of 1:100

1 100 µL 2D-LC sample (1:10) + 900 µL dilution solvent = 1000 µL (1:100)

Dilution of the 2D-LC starter sample in a ratio of 1:1000

1 100 µL 2D-LC sample (1:100) + 900 µL dilution solvent = 1000 µL (1:1000)

NOTE For the 2D-LC Addon Software Solution please refer to the User Manual of the Addon Software.

Run the Experiment

Run the Checkout Procedure for Multiple Heart-Cutting (2D-LC)

To run the checkout, various hardware configurations are possible, see Table 8 on page 54. Not all options can be shown. As example the Table 25 on page 234 is used here.

The following parameters have been optimized for this standard configuration. Parameters can deviate slightly for your system. Run the experiment and subsequently edit or optimize the method for your setup.

Table 26: Recommended conditions in ^{1}D (HPLC) for MHC 2D-LC

Parameter	Value
^{1}D Column Compartment (MCT)	
Column	InfinityLab Poroshell 120 SB-C18, 2.1 x 100 mm, 2.7 µm (685775-902)
Column Temperature	40 °C
Stop Time	As pump/No limit
^{1}D Pump	
Mobile Phase A	Water + 0.2 % formic acid
Mobile Phase B	Methanol
Flow Rate	0.5 mL/min
Mobile Phase Gradient:	0 min - 45 % B 7 min - 54 % B 8 min - 90 % B
Autosampler	
Injection Volume	2 µL for Standard LC 1:10 0.5 µL Positive Mode for LCMS, 1:100 or 1:1000 depending on the used LCMS
Injection Needle Wash	In Flush Port, 10 s, acetonitrile/water (50/50) or alternative methanol/ water (50/50)
Stop Time	As pump/No limit
^{1}D Detector (DAD)	

Parameter	Value
Diode-array Detector Signal A	254 nm, 4 nm BW; Ref 360 nm, 100 nm BW Signal peak width 20 Hz
Stop Time	Stop time As pump/No limit
Peak Trigger	
Peak Detection Mode	Threshold
Threshold	100 mAU For UV system with 1:10 sample, adjust the threshold accordingly for other samples

Table 27: Recommended conditions in ^{2}D (HPLC) for Multiple Heart-Cutting

Parameter	Value
2D-LC Valve	
	MHC with 40 µL sample, Transfer Capillary, ASM Factor No
^{2}D Column Compartment (MCT)	
Column	Poroshell 120 Bonus-RP, 3.0 x 50 mm, 2.7 µm (699968-301)
Column Temperature	40 °C
Stop Time	As pump/No limit
^{2}D Pump	
Operation Mode	Heart Cutting (peak based)
Mobile Phase A	Water + 0.2 % formic acid
Mobile Phase B	Acetonitrile
Flow Rate	1 mL/min
Idle Flow	not used
Stop Time	10 min (will not automatically prolonged, if peaks in 2D are not work off)
Post Time	3 min
Sampling Table	2.7 min, Start Peak-based, MHC Sampling time: 6 s 3.7 min, End Peak-based The Cut-Time (MHC) can vary slightly depending on the configuration and the used hardware.
^{2}D Cycle Time	Analysis 1.50 min, Equilibration 0.70 min
^{2}D Gradient	0 min - 10 % B Shift 7 min - 30 % B 1.50 min - 60 % B

Parameter	Value
Flush Gradient	0 min - 10 % B 0.05 min - 80 % B 0.8 min - 80 % B Duration: 0.8 min, Equilibration: 0.7 min
²D Detector (DAD)	
Diode-array	254 nm, 4 nm BW; Ref 360 nm, 100 nm BW Signal peak width 80 Hz
Stop Time	As pump/No limit

Table 28: Recommended conditions in ²D (LC-MS)

Parameter	Value
Ion Source	Atmospheric pressure electrospray (Dual AJS ESI) [6]
Ion Mode	Dual AJS ESI
Ion Polarity	Positive
Storage Mode	Both, Centroid preferred
LCMS Stream	MS
Acquisition Mode	Acquisition Mode MS1 Min Range (m/z) 50 , Max Range (m/z) 500 , Scan Rate (spectra/sec) 3
Instrument Parameters	**Source Parameters**
Gas Temp	250 °C
Gas Flow	11 L/min
Nebulizer	40 psig
SheathGasTemp	350 °C
SheathGasFlow	12 L/min
Scan Segment	1
	Scan Source Parameters
Vcap	3500 V
Nozzle Voltage	300 V
Fragmentor	120
Skimmer1	45
OctopoleRFPeak	750

6 For other ion sources than Dual AJS ESI the flow rate may need to be adjusted

Parameter	Value
	ReferenceMasses
Ref Mass Enabled	Enabled
Use Bottle A RefNebulizer	True
Ref Nebulizer	0 psig
	AutoRecalibration
Average Scans	1
Detection Window (ppm)	100 ppm
Min Height	1000 counts
	Reference Masses
	Positive
	121.05087300
	922.00979800
	Chromatograms
	Chrom Type Label Offset Y-Range
	TIC TIC 15 10000000
	TIC TIC 15 10000000
Stop Time	As pump/No limit

Table 29: Recommended conditions in ^{2}D (LC-MS) - SQ MS

Parameter	Value
ESI Source Parameter	Similar to the High-end MS parameter
Peak Width	0.06 min
SCAN	100 – 500 m/z in positive mode
Dwell Time	200 ms

1 Load method **Multiple Heart-Cutting Checkout** from the 2D-LC data media and modify the settings for your multiple heart-cutting configuration.

2 Run the method with 5190-6895 (2D-LC starter sample, 1 x 2 mL) , 1:10 (for only UV Checkout), 1:100 (for LCMS Checkout), or 1:1000 (for LCMS Checkout) diluted with Methanol/Water (20/80; v/v) with 0.1 % formic acid.

3 If necessary, subsequently edit or optimize the method.

Run the Checkout Procedure for High-Resolution (LC-LC)

To run the checkout, various hardware configurations are possible, see Table 8 on page 54. Not all options can be shown. As example the Table 25 on page 234 is used here.

The following parameters have been optimized for this standard configuration. Parameters can deviate slightly for your system. Run the experiment and subsequently edit or optimize the method for your setup.

Table 30: Recommended conditions in ^{1}D (HPLC) for HiRes 2D-LC

Parameter	Value
^{1}D Column Compartment (MCT)	
Column	InfinityLab Poroshell 120 SB-C18, 2.1 x 100 mm, 2.7 µm (685775-902)
Column Temperature	40 °C
Stop Time	As pump/No limit
^{1}D Pump	
Mobile Phase A	Water + 0.2 % formic acid
Mobile Phase B	Methanol
Flow Rate	0.5 mL/min
Mobile Phase Gradient:	0 min - 45 % B 7 min - 54 % B 8 min - 90 % B 10 min - 90 % B 10.1 min - 45 % B
Autosampler	
Injection Volume	2 µL for Standard LC 1:10 0.5 µL Positive Mode for LCMS, 1:100 or 1:1000 depending on the used LCMS
Injection Needle Wash	In Flush Port, 10 s, acetonitrile/water (50/50) or alternative methanol/water (50/50)
Stop Time	As pump/No limit
^{1}D Detector (DAD)	
Diode-array Detector Signal A	254 nm, 4 nm BW; Ref 360 nm, 100 nm BW Signal peak width 20 Hz
Stop Time	Stop time As pump/No limit
Peak Trigger	

Parameter	Value
Peak Detection Mode	Threshold
Threshold	100 mAU

Table 31: Recommended conditions in ^{2}D (HPLC) for HiRes 2D-LC

Parameter	Value
2D-LC Valve	
	MHC with 40 µL sample, Transfer Capillary, ASM Factor No
^{2}D Column Compartment (MCT)	
Column	Poroshell 120 Bonus-RP, 3.0 x 50 mm, 2.7 µm (699968-301)
Column Temperature	40 °C
Stop Time	As pump/No limit
^{2}D Pump	
Operation Mode	Heart Cutting (time-based)
Mobile Phase A	Water + 0.2 % formic acid
Mobile Phase B	Acetonitrile
Flow Rate	1 mL/min
Idle Flow	not used
Stop Time	18 min (will not automatically prolonged, if peaks in 2D are not work off)
Post Time	off
Sampling Table	3.22 min, Time-based Heart Cut, HiRes 5 x 3.8 s. LoopFill 79 % The Cut-Time (HiRes) can vary slightly depending on the configuration and the used hardware.
^{2}D Cycle Time:	Analysis 1.50 min, Equilibration 0.70 min
^{2}D Gradient:	0 min - 10 % B Shift 7 min - 30 % B 1.50 min - 60 % B
Flush Gradient	0 min - 10 % B 0.05 min - 80 % B 0.8 min - 80 % B Duration: 0.8 min, Equilibration: 0.7 min
^{2}D Detector (DAD)	
Diode-array	254 nm, 4 nm BW; Ref 360 nm, 100 nm BW Signal peak width 80 Hz
Stop Time	As pump/No limit

Table 32: Recommended conditions in ^{2}D (LC-MS)

Parameter	Value
Ion Source	Atmospheric pressure electrospray (Dual AJS ESI) [7]
Ion Mode	Dual AJS ESI
Ion Polarity	Positive
Storage Mode	Both, Centroid preferred
LCMS Stream	MS
Acquisition Mode	Acquisition Mode MS1 Min Range (m/z) 50 , Max Range (m/z) 500 , Scan Rate (spectra/sec) 3
Instrument Parameters	**Source Parameters**
Gas Temp	250 °C
Gas Flow	11 L/min
Nebulizer	40 psig
SheathGasTemp	350 °C
SheathGasFlow	12 L/min
Scan Segment	1
	Scan Source Parameters
Vcap	3500 V
Nozzle Voltage	300 V
Fragmentor	120
Skimmer1	45
OctopoleRFPeak	750
	ReferenceMasses
Ref Mass Enabled	Enabled
Use Bottle A RefNebulizer	True
Ref Nebulizer	0 psig
	AutoRecalibration
Average Scans	1
Detection Window (ppm)	100 ppm

7 For other ion sources than Dual AJS ESI the flow rate may need to be adjusted

Parameter	Value
Min Height	1000 counts
	Reference Masses
	Positive
	121.05087300
	922.00979800
	Chromatograms
	Chrom Type Label Offset Y-Range
	TIC TIC 15 10000000
	TIC TIC 15 10000000
Stop Time	As pump/No limit

Table 33: Recommended conditions in ^{2}D (LC-MS) - SQ MS

Parameter	Value
ESI Source Parameter	Similar to the High-end MS parameter
Peak Width	0.06 min
SCAN	100 – 500 m/z in positive mode
Dwell Time	200 ms

1. Load method **High-Resolution Checkout** from the 2D-LC data media and modify the settings for your multiple heart cutting configuration.
2. Run the method with 5190-6895 (2D-LC starter sample, 1 x 2 mL) , 1:10 (for only UV Checkout), 1:100 (for LCMS Checkout), or 1:1000 (for LCMS Checkout) diluted with Methanol/Water (20/80; v/v) with 0.1 % formic acid.
3. If necessary, subsequently edit or optimize the method.

Run the Checkout Procedure for Comprehensive (LCxLC)

To run the checkout, various hardware configurations are possible, see Table 8 on page 54. Not all options can be shown. As example the Table 25 on page 234 is used here.

To achieve optimal sensitivity, in comprehensive mode, especially for LC/MS applications, the LC flow is often split prior to the mass spectrometer.

The following parameters have been optimized for this standard configuration. Parameters can deviate slightly for your system. Run the experiment and subsequently edit or optimize the method for your setup.

Table 34: Example for a MS passive splitter setup (ratio 1:2)

Description (PN)	Usage
Tee, Zero 1/16"SS Low dead volume (0100-0969)	T-piece
5067-4659 (5067-4659)	^{2}D detector connected to T-piece
5500-1205 (5500-1205)	Inlet of the LCMS source connected to the other end of the T-piece
5500-1206 (5500-1206)	Remaining connection to the T-piece is used as waste capillary

Table 35: Recommended conditions in ^{1}D (HPLC) for comprehensive 2D-LC

Parameter	Value
^{1}D Column Compartment (MCT)	
Column	InfinityLab Poroshell 120 SB-C18, 2.1 x 100 mm, 2.7 µm (685775-902)
Column Temperature	40 °C
Stop Time	As pump/No limit
^{1}D Pump	
Mobile Phase A	Water + 0.2 % formic acid
Mobile Phase B	Methanol
Flow Rate	0.1 mL/min
Stop Time	40 min

Parameter	Value
Post Time	10 min
Mobile Phase Gradient:	0 min - 40 % B 34 min - 60 % B 34.5 min - 90 % B 40 min - 90 % B
Autosampler	
Injection Volume	2 µL for Standard LC 0.5 µL Positive Mode for LCMS
Injection Needle Wash	In Flush Port, 10 s, methanol/water (50/50)
Stop Time	As pump/No limit
^{1}D Detector (DAD)	
Diode-array Detector Signal A	254 nm, 4 nm BW; Ref 360 nm, 100 nm BW Signal peak width 20 Hz
Stop Time	Stop time As pump/No limit

Table 36: Recommended conditions in ^{2}D (HPLC) for comprehensive 2D-LC

Parameter	Value
2D-LC Valve	
	2D-LC valve with 40 µL sample loop (or with 60 µL sample loop)
^{2}D Column Compartment (MCT)	
Column	Poroshell 120 Bonus-RP, 3.0 x 50 mm, 2.7 µm (699968-301)
Column Temperature	50 °C
Stop Time	As pump/No limit
^{2}D Pump	
Operation Mode	Comprehensive
Mobile Phase A	Water + 0.2 % formic acid
Mobile Phase B	Acetonitrile
Flow Rate	2.5 mL/min
Idle Flow	0.3 mL/min
Stop Time	40 min
Post Time	10 min
Sampling Table	Start 5 min, Stop at 40 min s. LoopFill 80 %

Parameter	Value
^{2}D Cycle Time	Analysis 0.25 min (0.35 min if 60 µL sample loop is used), Equilibration 0.10 min
^{2}D Gradient:	0 min - 5 % B 0.25 min (0.35 min if 60 µL sample loop is used) - 95 % B
^{2}D Detector (DAD)	
Diode-array	254 nm, 4 nm BW; Ref 360 nm, 100 nm BW Signal peak width 80 Hz
Stop Time	As pump/No limit

Table 37: Recommended conditions in ^{2}D (LC-MS)

Parameter	Value
Ion Source	Atmospheric pressure electrospray (Dual AJS ESI)
Ion Mode	Dual AJS ESI
Ion polarity	Positive
Storage Mode	Both, Centroid preferred
LCMS Stream	MS
Acquisition Mode	Acquisition Mode MS1 Min Range (m/z) 50 , Max Range (m/z) 500 , Scan Rate (spectra/sec) 3
Instrument Parameters	**Source Parameters**
Gas Temp	250 °C
Gas Flow	11 L/min
Nebulizer	40 psig
SheathGasTemp	350 °C
SheathGasFlow	12 L/min
Scan Segment	1
	Scan Source Parameters
Vcap	3500 V
Nozzle Voltage	300 V
Fragmentor	120
Skimmer1	45
OctopoleRFPeak	750
	ReferenceMasses
Ref Mass Enabled	Enabled

Parameter	Value
Use Bottle A RefNebulizer	True
Ref Nebulizer	0 psig
	AutoRecalibration
Average Scans	1
Detection Window (ppm)	100 ppm
Min Height	1000 counts
	Reference Masses
	Positive
	121.05087300
	922.00979800
	Chromatograms
	Chrom Type Label Offset Y-Range
	TIC TIC 15 10000000
	TIC TIC 15 10000000
Stop Time	As pump/No limit
To avoid problems in the LC/MS due to the high flow rate, the effluent from the second dimension column should be split. The recommended split ratio is 1:2	

Table 38: Recommended conditions in ^{2}D (LC-MS) - SQ MS

Parameter	Value
ESI Source Parameter	Similar to the High-end MS parameter
Peak Width	0.06 min
SCAN	100 – 500 m/z in positive mode
Dwell Time	200 ms

1 Load method **Comprehensive Checkout** from the 2D-LC data media and modify the settings for your **Comprehensive** configuration.

2 Run the method with 5190-6895 (2D-LC starter sample, 1 x 2 mL) , 1:10 (for only UV Checkout), 1:100 (for LCMS Checkout), or 1:1000 (for LCMS Checkout) diluted with Methanol/Water (20/80; v/v) with 0.1 % formic acid.

3 If necessary, subsequently edit or optimize the method.

9 Data Analysis

This chapter provides information on how to analyze 2D-LC data with software.

2D-LC Data Analysis/Data Evaluation for MassHunter

Presets in MassHunter Acquisition

For better data analysis of the Multiple Heart-Cutting or High-Resolution Sampling, an extra selection step is required in the data acquisition. This measure will order the generated ^{2}D cuts correctly, which will facilitate the display and data analysis later on.

Comprehensive 2D-LC measurements can be displayed and analyzed with GC Image LCxLC Edition Software.

For further details, see

- Data Analysis for Comprehensive 2D-LC (LCxLC)
- The online help of GC Image LCxLC Edition Software
- www.gcimage.com

Automated File Splitting

To automatically generate the correct cutting sequence after each 2D-LC measurement, in the Method Editor start the 2D-LC File Splitter Automation function.

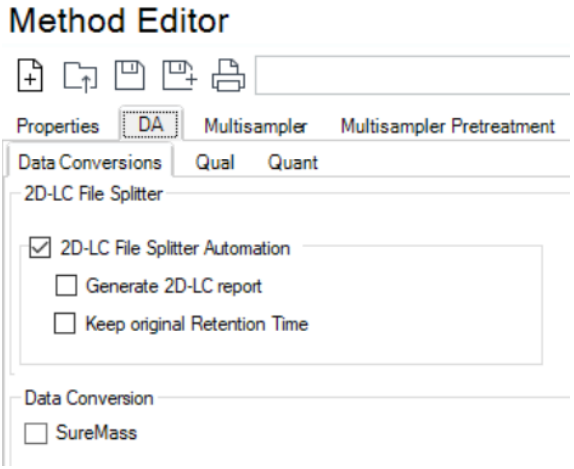


Figure 143: Method Editor in MassHunter Acquisition

1 Select the check box 2D-LC File Splitter Automation in Method Editor > DA .

This selection will automatically generate the correct cutting sequence after each 2D-LC measurement.

2 **Optional:** Select the check box Generate 2D-LC report.

This selection will generate a special pdf 2D-LC report with cut info in the data folder.

3 **Optional:** Select the check box Keep original Retention Time.

This selection will keep the information on the retention time from the ^{1}D run.

4 For Single Sample Runs

In addition to start the file splitting process for a single sample run you need.

- In Method part to run:

  Both Acquisition and DA must be selected.

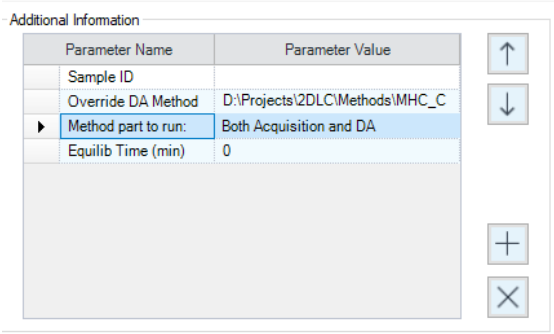


Figure 144: Additional Information view of Single Sample runs

- In Override DA method:

  Define the path where the Acquisition methods with activated File Splitter Automation are stored.

NOTE

The 2D-LC File Splitter automation is limited to two detectors (UV and MS detector) in the second dimension. If more than two second dimension detectors are configured, the UV detector with the shortest delay (transfer volume) is used for splitting.

In case it was forgotten to activate DA:

Re-run sample / worklist with DA-Reprocessing Tool.

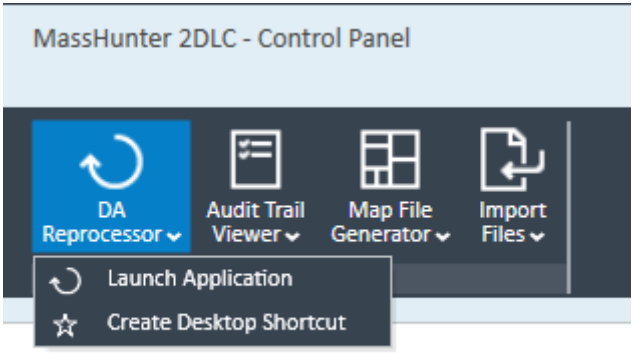


Figure 145: DA Reprocessor view in the Control Panel

This function is automatically installed with the Acquisition Software and can be found in **Control Panel** (under option **Tools**)

Separately it is available from the *Offline Utilities* DVD.

MassHunter 2D-LC File Structure

The 2D-LC results from the MassHunter Acquisition have a special data structure. In the example shown, the 2D-LC data are analyzed with an LC/MS UV-QTOF instrument and evaluated with the MassHunter Qualitative Analysis Software 10.0.

2D-LC_File.d	This file stores the complete information from ^{2}D run, e.g the MS, and the UV signals.
2D-LC Folder_Cuts	This folder stores and lists all cuts in the correct order by cut number and cut time, e.g **Filename – Cut01 at 2.31 min.d**.

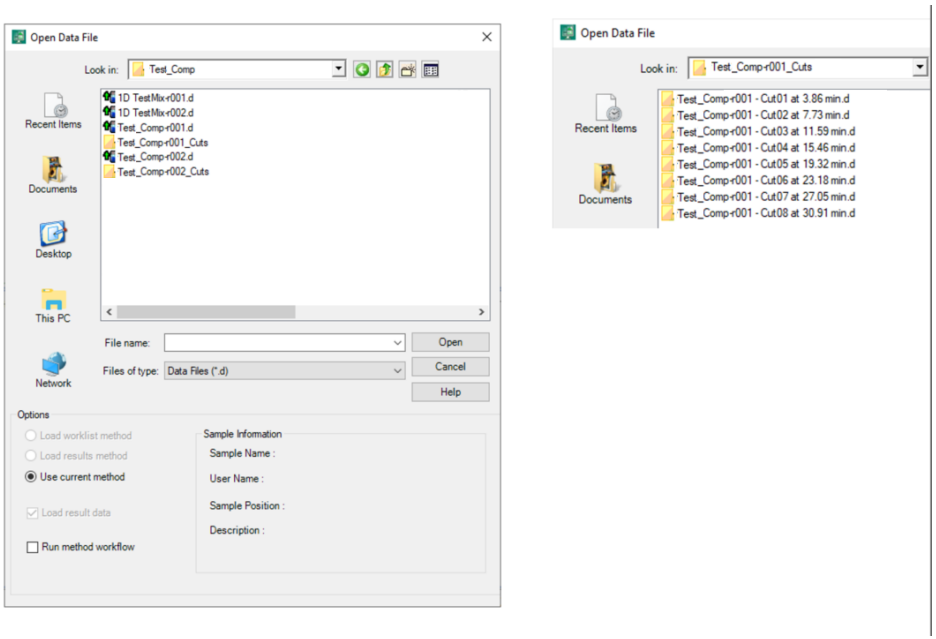


Figure 146: File structure in MassHunter with parent data files and corresponding folder w/ cuts

NOTE The data files store cut info as **cuts.csv** and the file splitter log file as **FSsplitterlog.txt**.

NOTE To avoid issues in the DA processing, for MassHunter 11 Workstation do not set up project names or file names containing blanks.

MassHunter Qualitative Analysis Software

The Masshunter data analysis software generally works with ^{2}D data in the same way as you are used to. The task to identify compounds or setup and run qualitative analysis methods can also be performed on 2D-LC data. However, to make it easier to get started with ^{2}D data, we have listed some different workflows as examples.

The 2D-LC instrument in this case was equipped with 2 UV detectors (^{1}D and ^{2}D), 2D-LC valve with MHC and a Q-TOF detector in the second dimension. Default method is loaded.

Workflow ^{1}D UV Data Extraction – Alternative 1

1 Open Data File **2D-LC file.d**, in this case **Test_Comp r001.d**.

The string of 2D TIC-chromatograms appears.

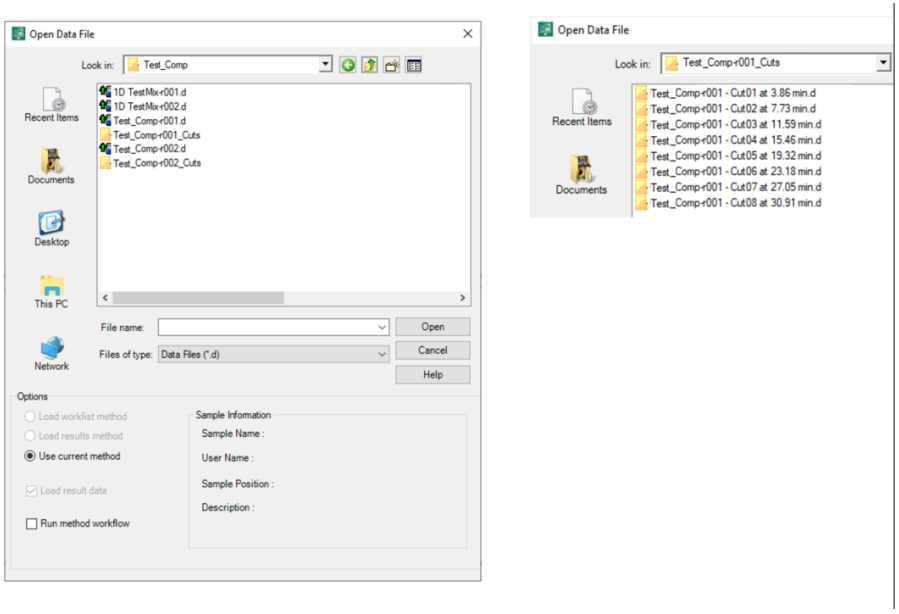


Figure 147: Open Data File view

2 Right-click Chromatogram Results and select Extract > Other Chromatograms > DAD 1 .

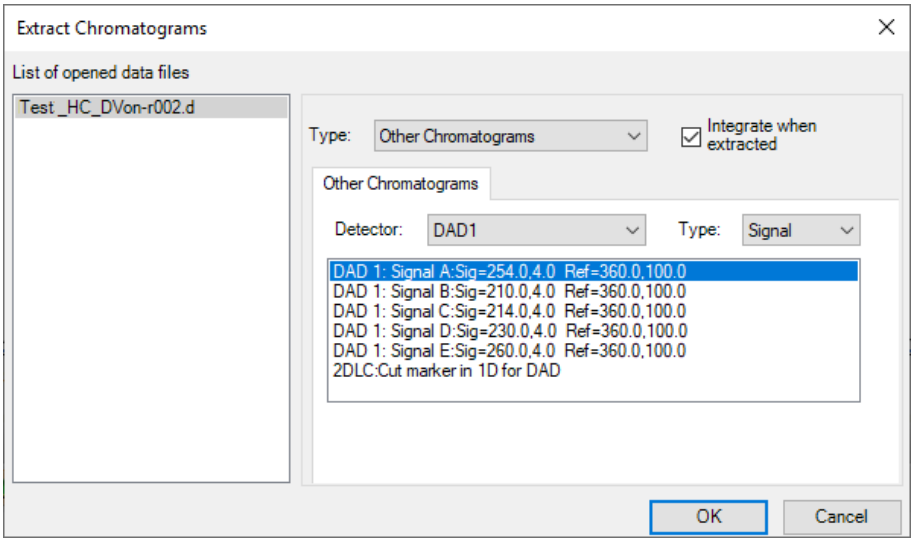


Figure 148: Extract Chromatograms view

3 Right-click Chromatogram Results and Extract > Other Chromatograms > 2DLC Cut marker in 1D for DAD .

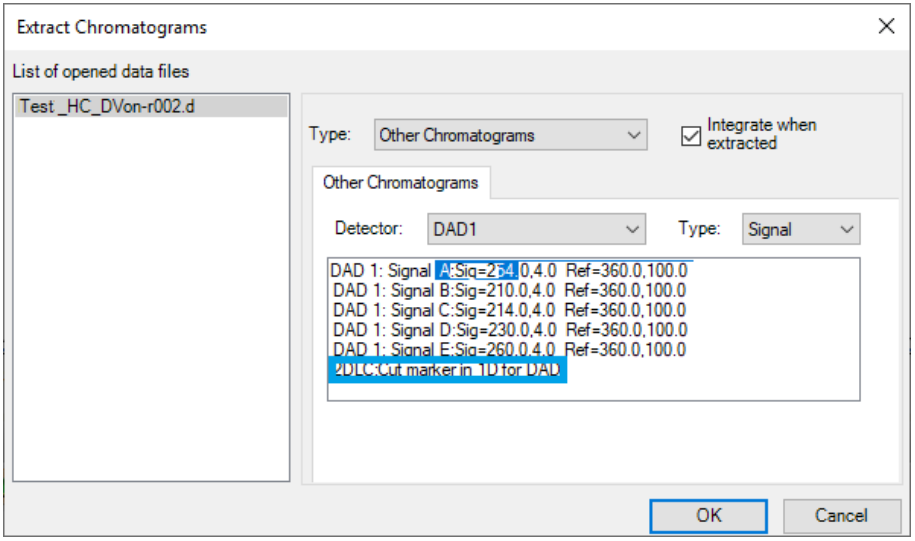


Figure 149: Extract Chromatograms view with selected 2DLC Cut marker

May be automated using Method Automation Workflow.

NOTE By default, the Agile2 integrator is chosen to integrate UV chromatograms. To Integrate cut markers, you have to use the “general” integrator. Thus, the specified times correspond to the cut signals generated by the DA.

4 Mark DAD 1 and cut marker and press **show highlighted signals** button.

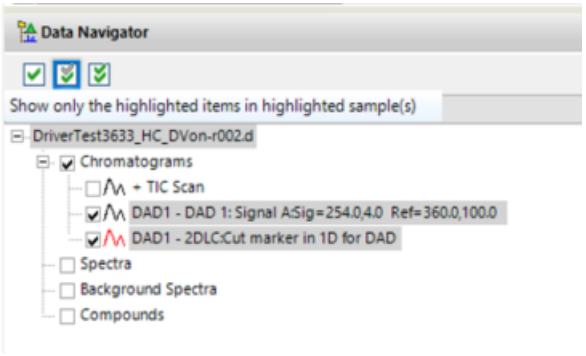


Figure 150: Data Navigator view

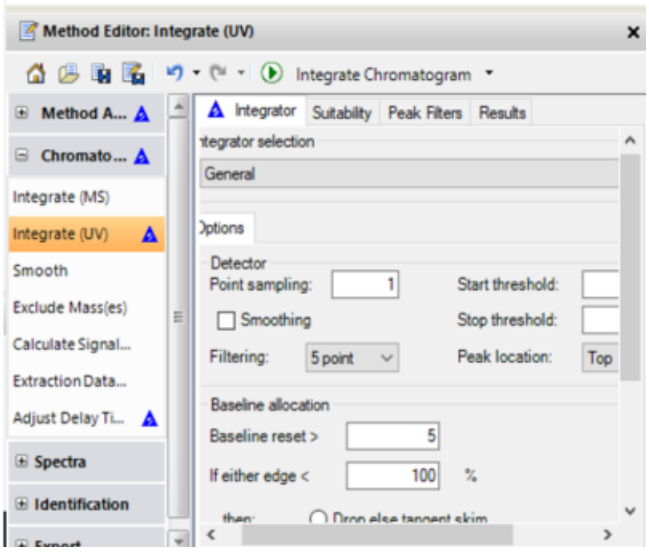


Figure 151: Method Editor Integrate (UV)

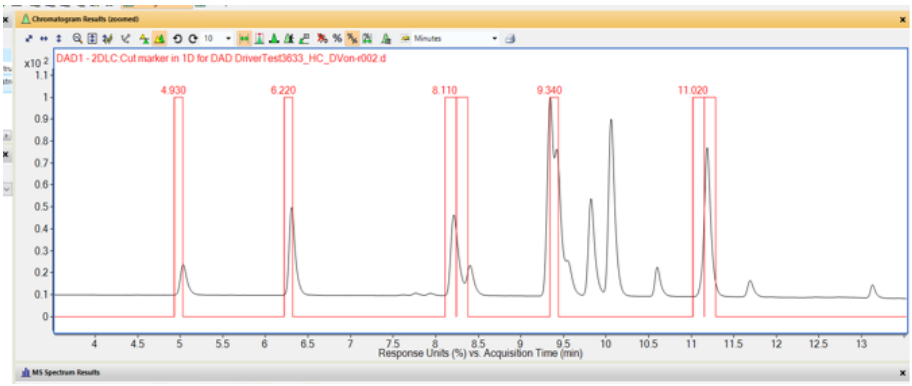


Figure 152: [1]D signal overlaid w/ cut marker

Workflow ^{1}D UV data extraction – Alternative 2

1 Open Data File **2D-LC file.d**, in this case **Test_Comp r001.d**.

The string of 2D TIC-chromatograms appears.

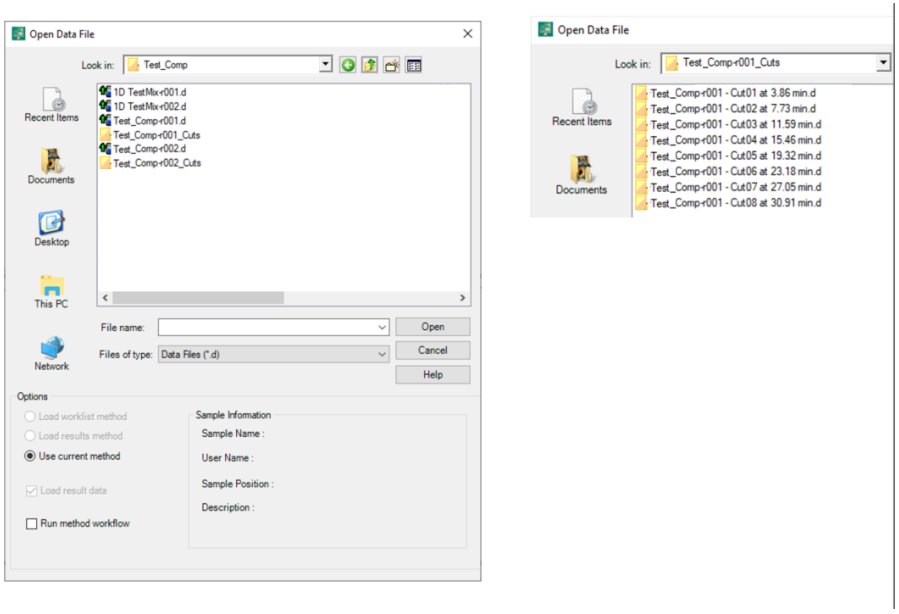


Figure 153: Open Data File view

2 Go to Actions and select **Extract All non-MS Chromatograms**.

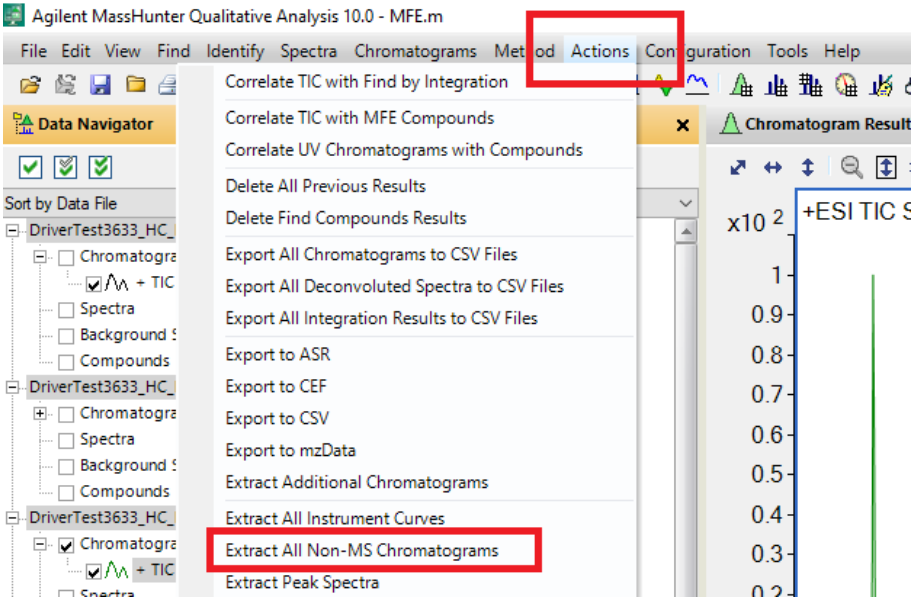


Figure 154: Actions menu view for Extract All Non-MS Chromatograms

3 Mark DAD 1 and cut marker and press **show highlighted signals** button.

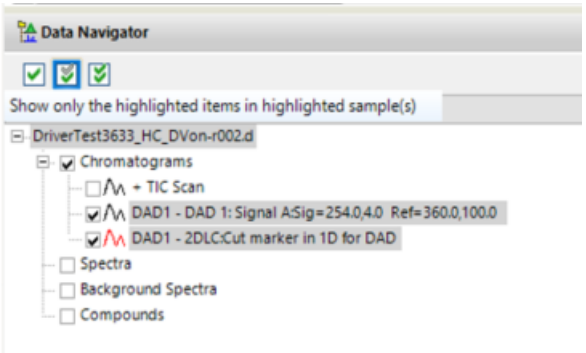


Figure 155: Data Navigator view

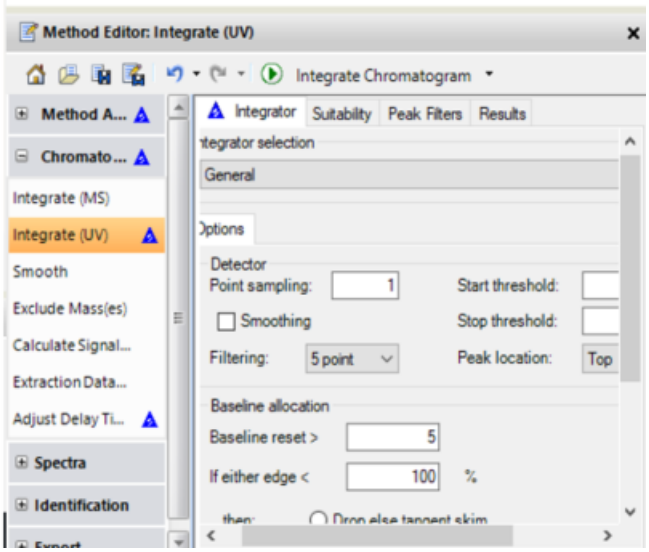


Figure 156: Method Editor Integrate (UV)

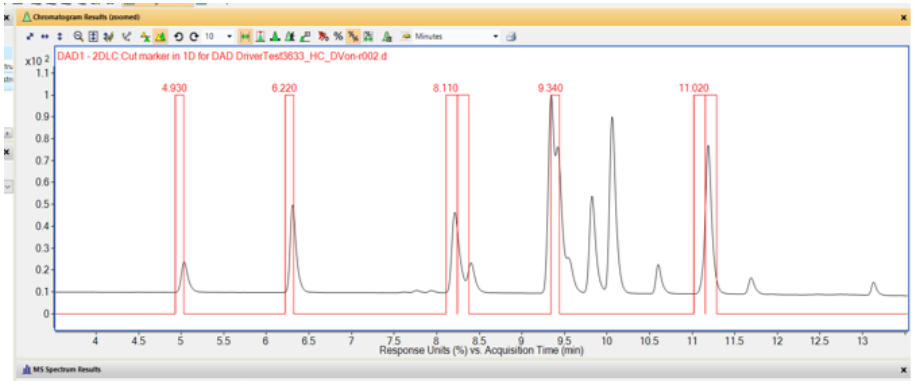


Figure 157: [1]D signal overlaid w/ cut marker

Workflow ^{2}D MS Data

1 Open “extracted 2D cuts” from cut folder.

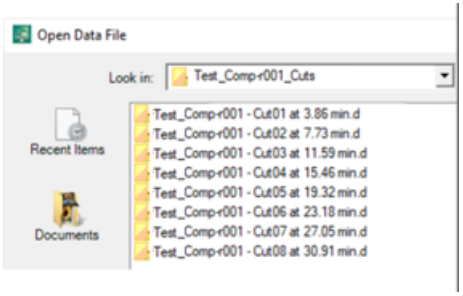


Figure 158: Open Data File view

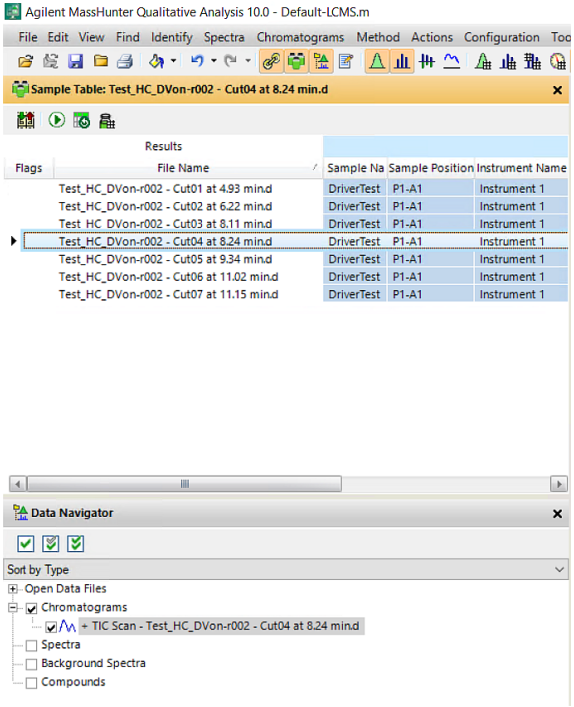


Figure 159: Select Chromatograms

^{2}D TIC-chromatograms appear in the Sample Table.

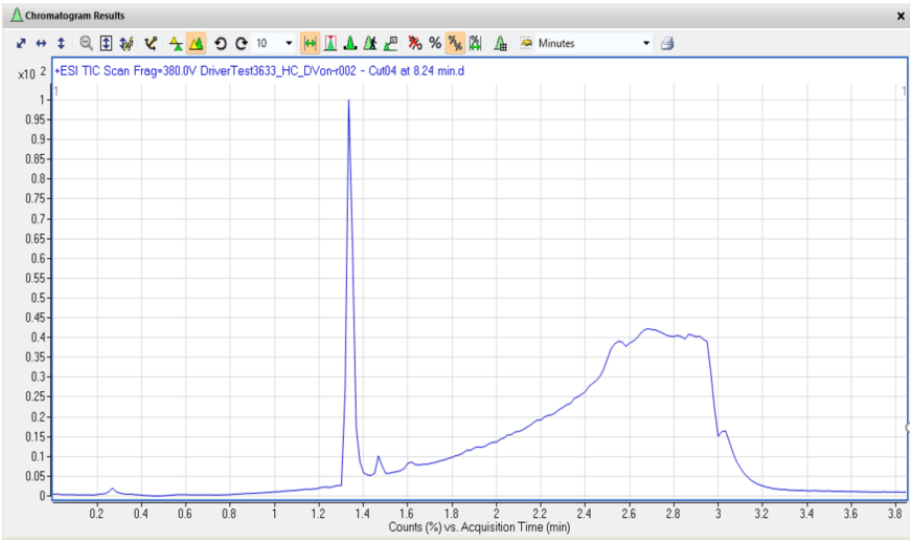


Figure 160: Chromatogram Results

2 Work with ^{2}D MS data as with ^{1}D data, e.g. ESI extraction.

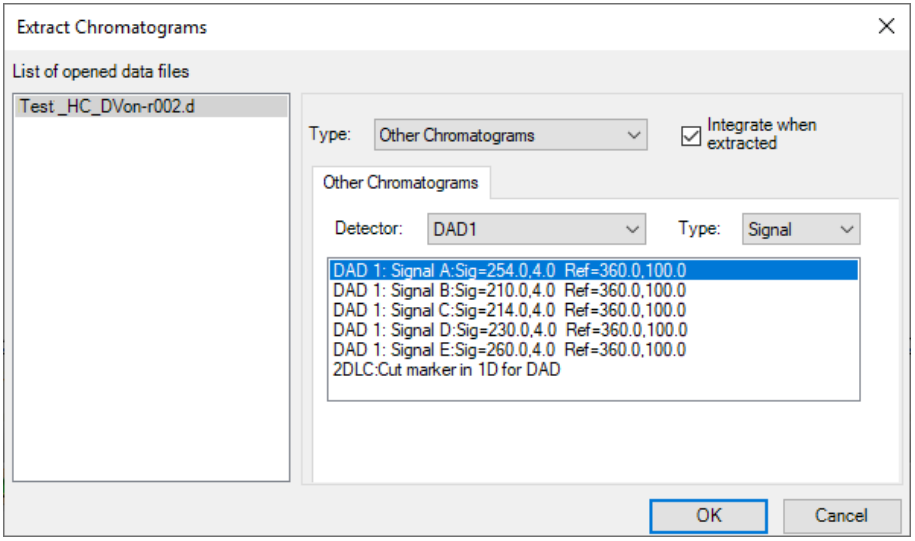


Figure 161: Extract Chromatograms view

NOTE Only one cut can be highlighted in the sample table for extraction purposes; highlighting several runs leads to an error in Qual.

Workflow Compare ^{2}D UV and MS data - Alternative 1

1 Load the 2D-LC experiment.

2 Mark a single cut in Sample Table.

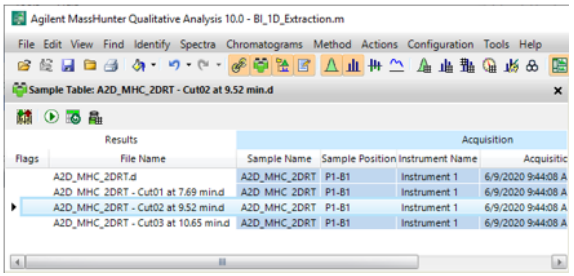


Figure 162: Sample Table

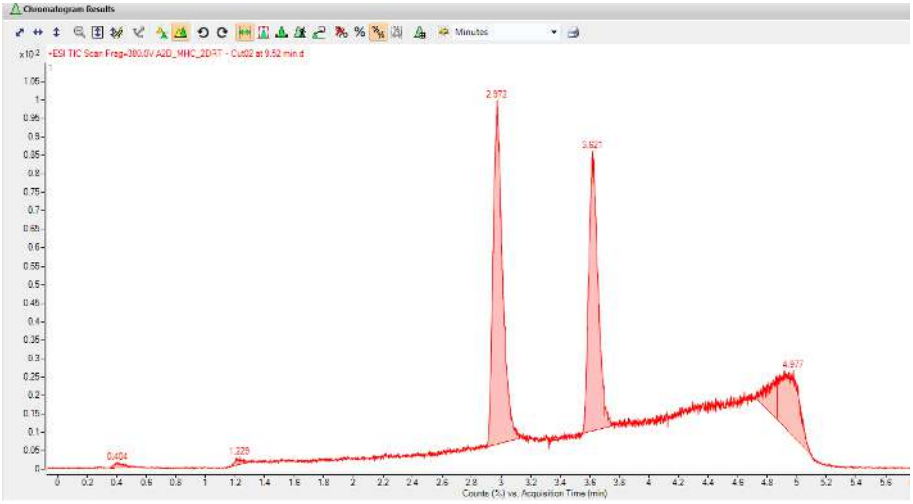


Figure 163: Chromatogram Result

3 Right-click **Chromatogram Results** and **Extract** > **Other Chromatograms** > **2D DAD signals** (those with "cut" in their name).

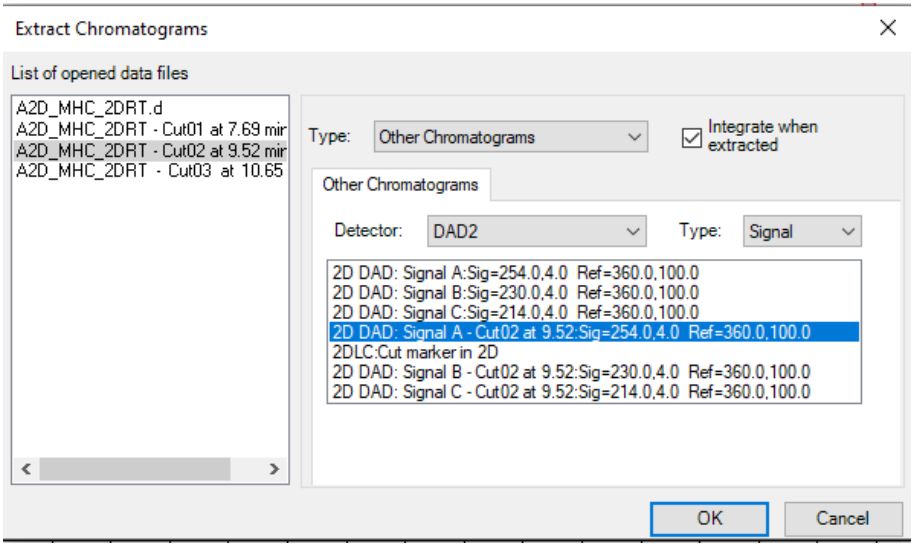


Figure 164: Chromatogram Results Cut02 at 9.52

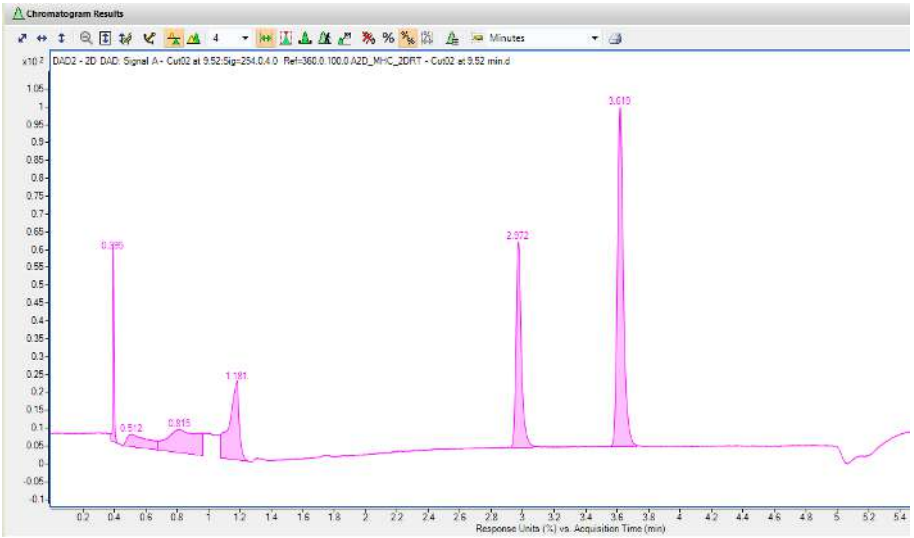


Figure 165: Extract Chromatograms 2D DAD Signal A Cut at 9.52

4 You may want to repeat with **2D-LC Cut Marker**, which gives an indication when each cut has been analyzed.

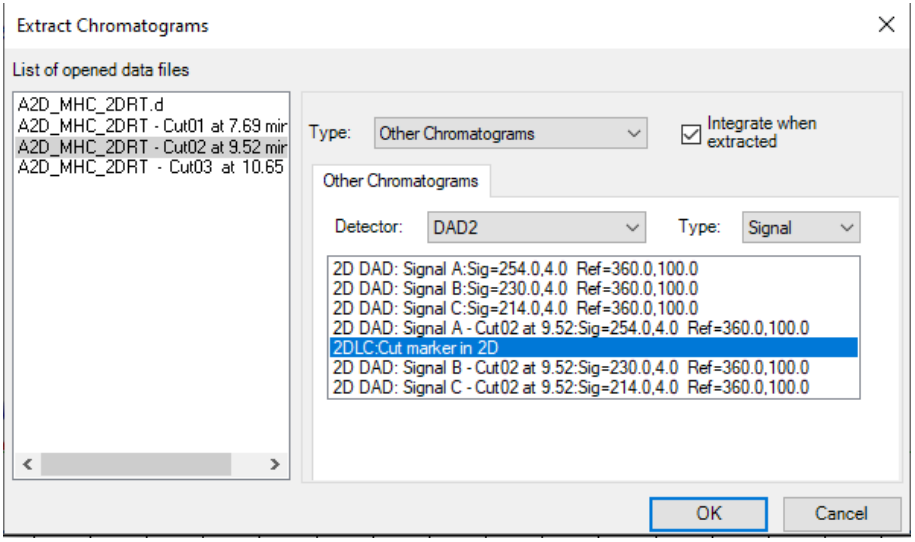
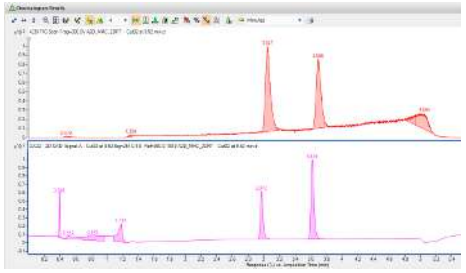


Figure 166: Extract Chromatograms 2DLC:Cut marker in 2D

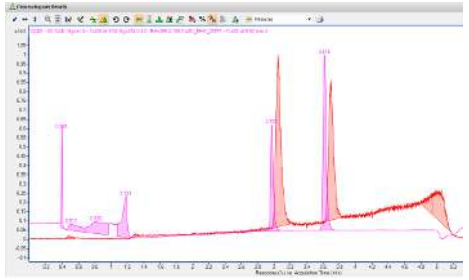
NOTE This cannot be automated because the name of the DAD trace has the cut # in it; thus cut #3 does not contain any data with a name of cut #2

NOTE Cut markers in ^{1}D shows the time when the cut was made. Cut markers in ^{2}D only makes sense, if you keep the retention time of each cut in method editor settings. Then you can verify which cut belongs to which chromatogram.

5 DAD data can now be compared with MS traces.



DAD Signal versus MS Signal



DAD Signal overlayed MS Signal

6 **Optional:** In case you want to shift chromatograms for alignment of UV and MS traces, use **Adjust Delay Time**.

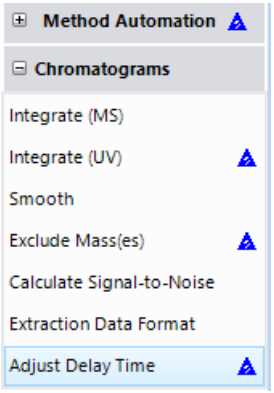


Figure 167: Adjust Delay Time

7 Then the retention time for MS1 and DAD2 was entered.

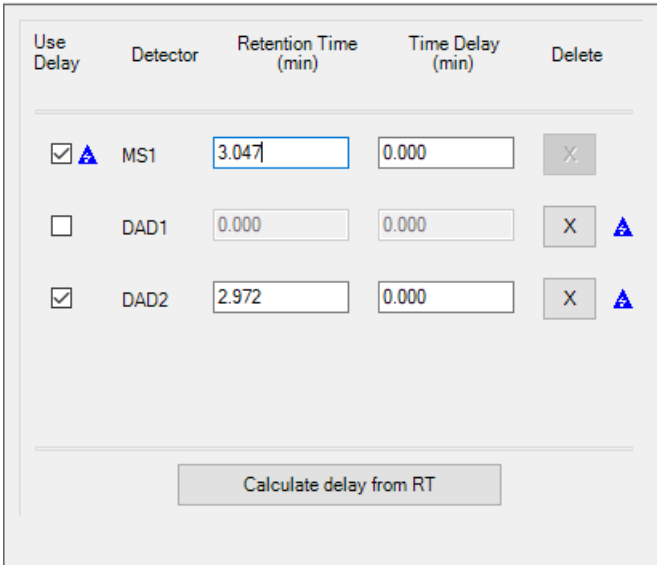


Figure 168: Retention Time Value for Peak1 (MS RT 3.047min and DAD2 2.972 min)

8 By pressing the **Calculate delay from RT** button and the delay time calculated at 0.075 min.

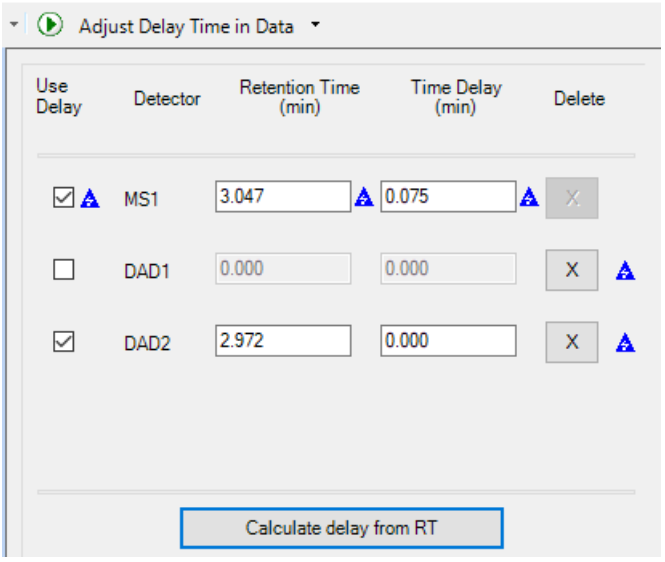


Figure 169: Delay time calculation

9 Press play button **Adjust Delay Time in Data** to align chromatograms.

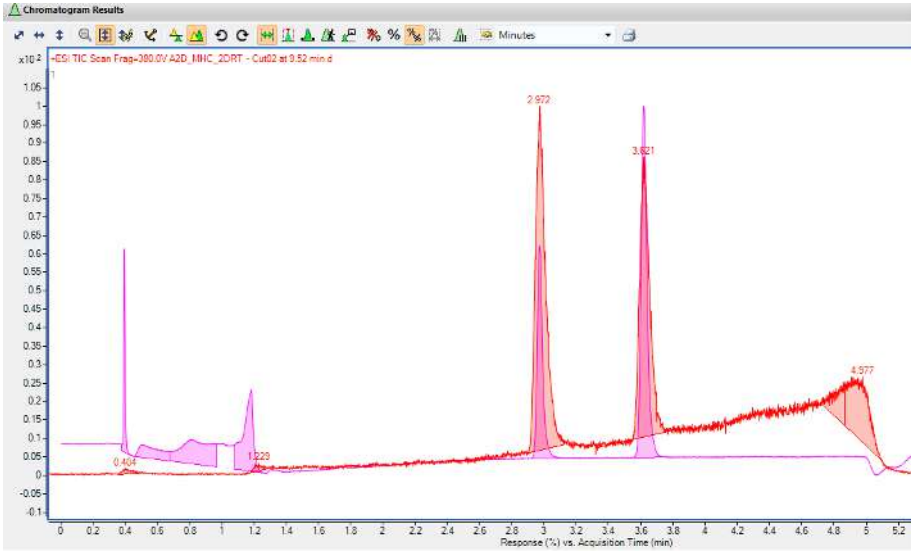


Figure 170: Overlay of the aligned two chromatograms

Workflow Compare ^{2}D UV and MS – Alternative 2

1 Load eight HiRes cuts from a 2D-LC High-Resolution experiment.

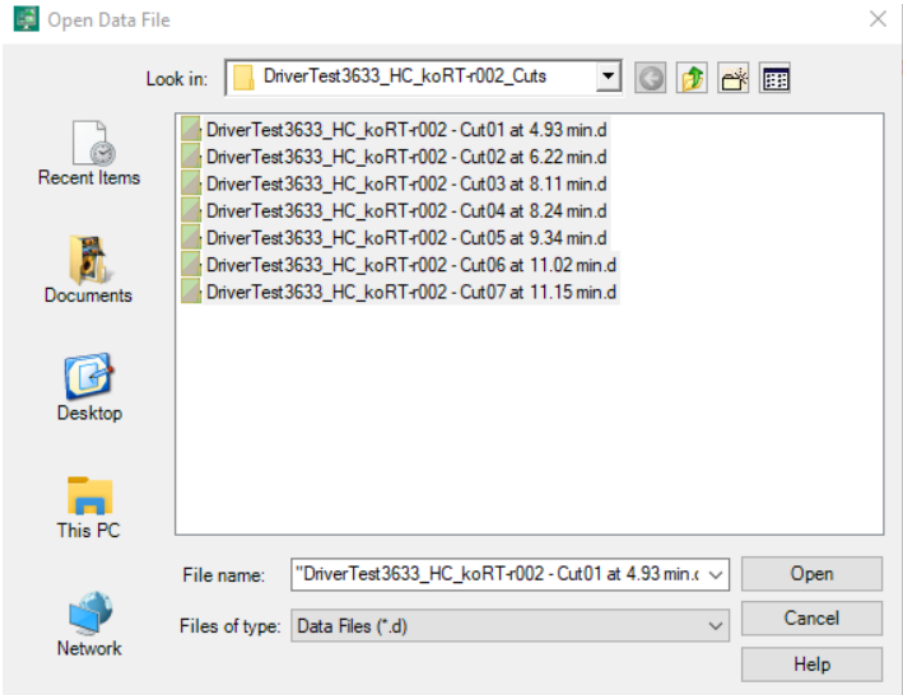
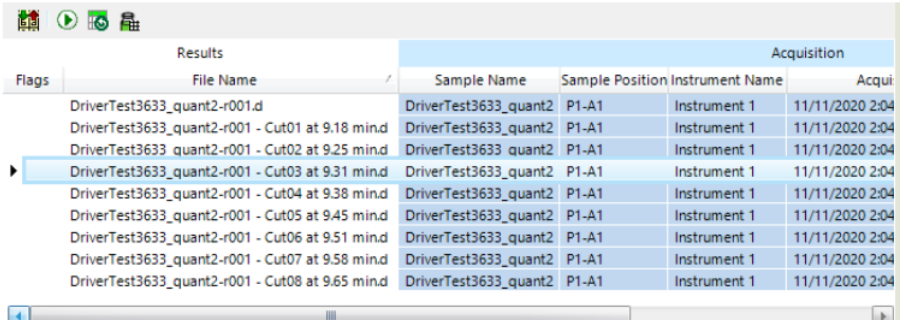


Figure 171: Files with results of the eight HiRes cuts



Results		Acquisition			
Flags	File Name	Sample Name	Sample Position	Instrument Name	Acqui
	DriverTest3633_quant2-r001.d	DriverTest3633_quant2	P1-A1	Instrument 1	11/11/2020 2:04
	DriverTest3633_quant2-r001 - Cut01 at 9.18 min.d	DriverTest3633_quant2	P1-A1	Instrument 1	11/11/2020 2:04
	DriverTest3633_quant2-r001 - Cut02 at 9.25 min.d	DriverTest3633_quant2	P1-A1	Instrument 1	11/11/2020 2:04
▶	DriverTest3633_quant2-r001 - Cut03 at 9.31 min.d	DriverTest3633_quant2	P1-A1	Instrument 1	11/11/2020 2:04
	DriverTest3633_quant2-r001 - Cut04 at 9.38 min.d	DriverTest3633_quant2	P1-A1	Instrument 1	11/11/2020 2:04
	DriverTest3633_quant2-r001 - Cut05 at 9.45 min.d	DriverTest3633_quant2	P1-A1	Instrument 1	11/11/2020 2:04
	DriverTest3633_quant2-r001 - Cut06 at 9.51 min.d	DriverTest3633_quant2	P1-A1	Instrument 1	11/11/2020 2:04
	DriverTest3633_quant2-r001 - Cut07 at 9.58 min.d	DriverTest3633_quant2	P1-A1	Instrument 1	11/11/2020 2:04
	DriverTest3633_quant2-r001 - Cut08 at 9.65 min.d	DriverTest3633_quant2	P1-A1	Instrument 1	11/11/2020 2:04

Figure 172: Sample Table of the HiRes cuts

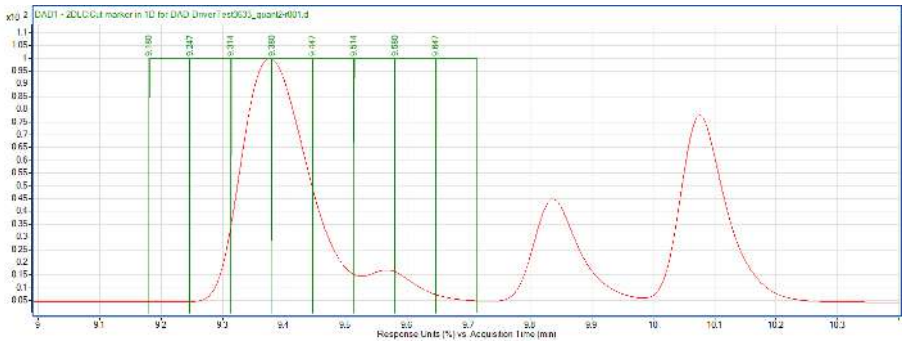


Figure 173: HiRes experiment

2 To extract the same EIC's across all cuts, highlight the EIC's and use the Use Highlighted Chromatograms > Extract from Data Files function.

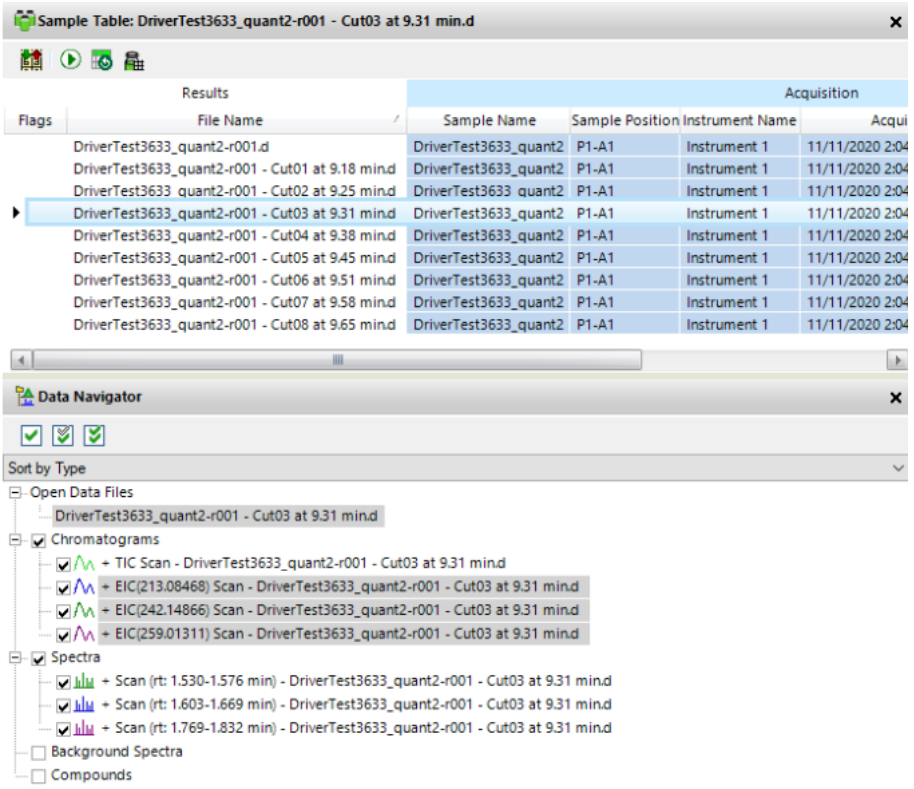


Figure 174: Extracted EIC chromatograms from one single cut

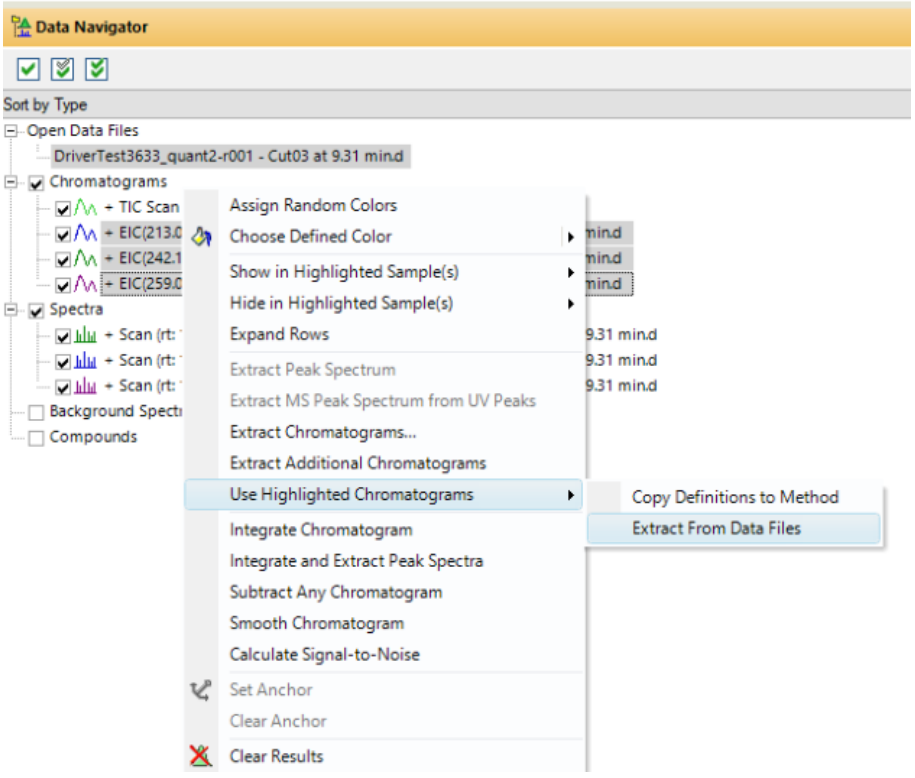


Figure 175: Highlighted chromatograms **Extract From Data Files** function

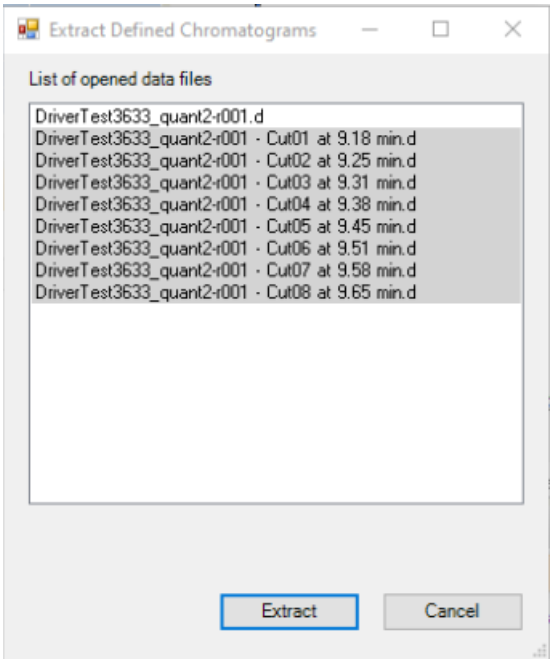
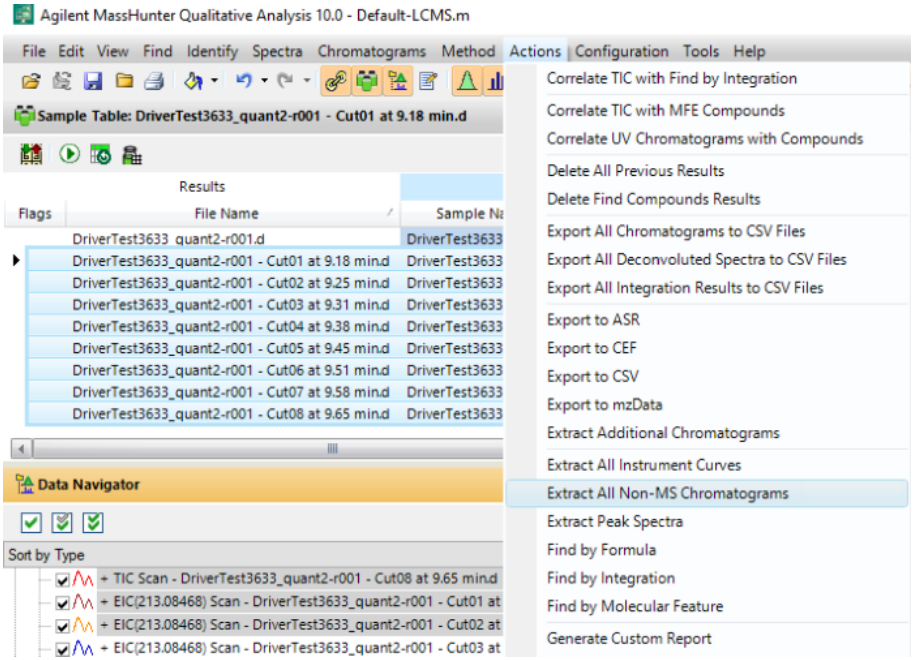


Figure 176: Extracted EIC chromatograms from all HiRes cuts

NOTE

The Use Highlighted ChromatogramsExtract from Data Files function is also accessible by right click on highlighted EIC data, or in Chromatograms Menu.

3 Mark ALL cuts in **Sample Table**. As with [1]D data, under Actions select **Extract All Non-MS Chromatograms**.



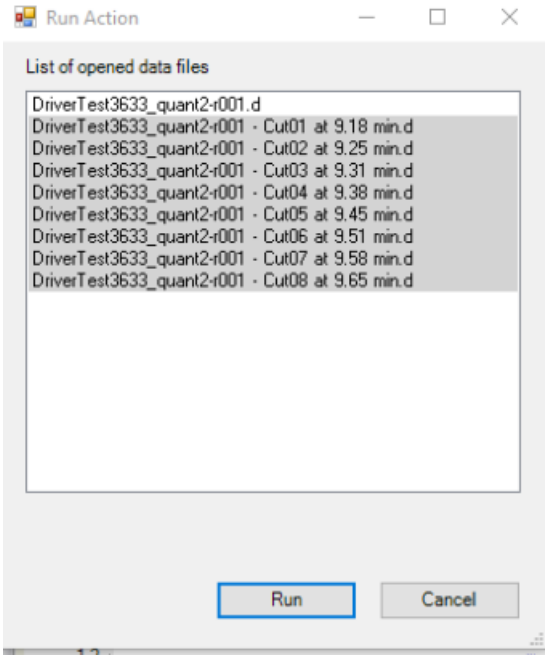


Figure 177: Selected cuts

4 In the Data Navigator, highlight the data to compare, and click show highlighted signals.

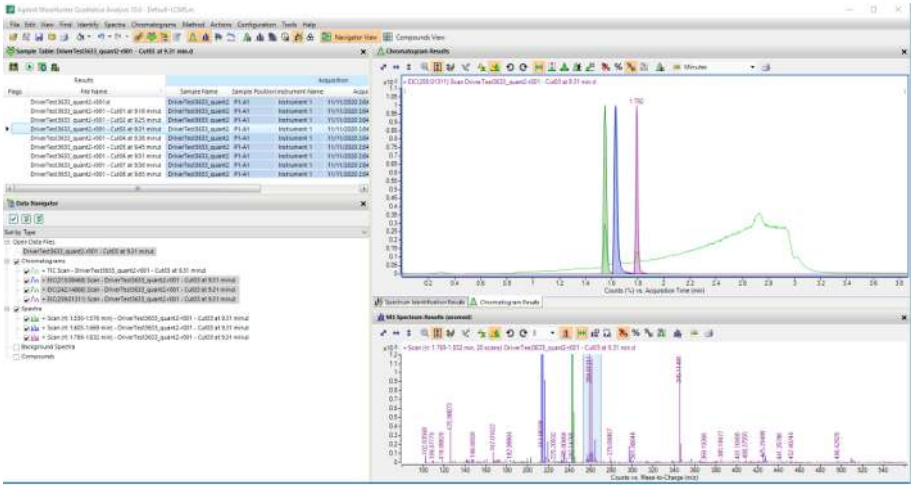


Figure 178: Comparison of Extracted EIC chromatograms

2D Data format: Keep original RT

If the check box **Keep original RT** is selected, the data displayed is relative to ^{1}D time scale, i.e. displayed when they were analyzed. This means that the original retention time from the DA of the acquisition method is retained, see Presets in MassHunter Acquisition on page 253.

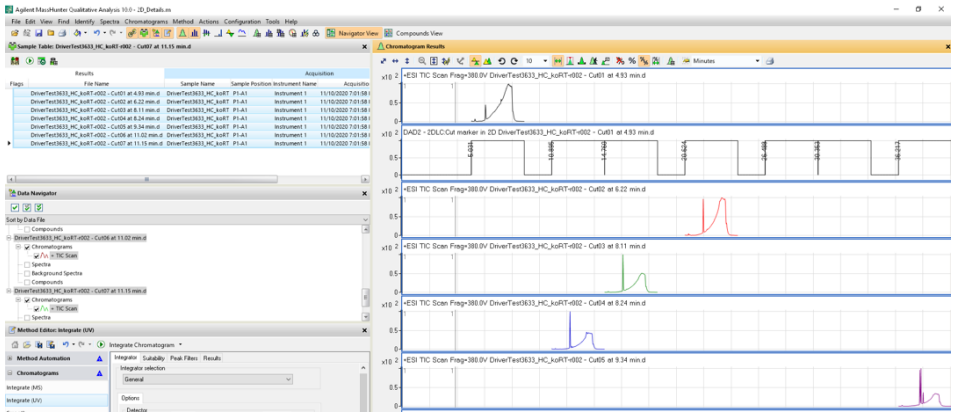


Figure 179: Example of Chromatogram Results where the original retention time is maintained

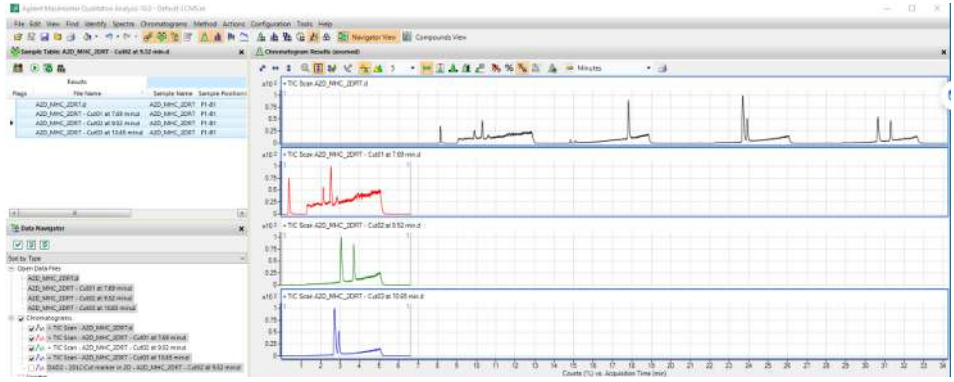


Figure 180: Example of Chromatogram Results with no original retention time

Data Analysis in MassHunter Quantitative Analysis Software

Quantitative data analysis of high-resolution sampling results can be carried out in both MH Quant and in GC Image software. In this section, MH Quant process is introduced.

1 Create batch.

Select the parent data files. Quantification targets can be either a ^{2}D UV signal or an extracted ion chromatogram (EIC), which are defined later in this method.

2 Define quantifier in the quant method.

In the full HiRes ^{2}D signal, the same compound appears multiple times at retention time intervals of ^{2}D cycle time. As such, the actual response of the compound should be represented by summation of individual peak response of the compound across the total number of cuts. To use the sum-up peak response approach, individual peaks from corresponding cut need to be defined as a unique compound. All such compounds are grouped under one same **Compound Group**. Then a pseudo-sum-up compound is defined and assigned to the same **Compound Group**. For this sum-up compound, select **Response Sum** from the **Compound Math** column drop-down list. See figure below as an example using a DAD2 signal to set up quantifiers for the compound prometryn.

Sample

Name	Data File	Type	Level	Acq. Method File	Acq. Date-Time
100FD	100FD.d	Cal		EasyStart_HiRe...	11/11/2021 3:1...

Quantifier

Name	Cmpd. Group	Signal Name	Signal Type	RT	Compound Math	Scan	Type	Product Ion
prometryn-cut4	1	DAD 2: Signal A	DAD	9.98		MS1 Scan	Target	0.0
prometryn-cut3	1	DAD 2: Signal A	DAD	12.13		MS1 Scan	Target	0.0
prometryn-cut2	1	DAD 2: Signal A	DAD	14.38		MS1 Scan	Target	0.0
prometryn-cut1	1	DAD 2: Signal A	DAD	16.58		MS1 Scan	Target	0.0
prometryn-Sum	1	DAD 2: Signal A	DAD	14.38	Response Sum	MS1 Scan	Target	0.0

Figure 181: Quantifier settings in MH Quant method based on 2D DAD signal. The compound prometryn appears in 4 out of 5 cuts at their respective RT in ^{2}D DAD signal channel. The **prometryn-Sum** is the sum-up compound whose response is the sum of individual compounds.

If MS-based quantification is desired, select **MS** as **Signal Type** and define the value of target ion in **Product Ion** column as the figure below shows.

Quantifier

Name	Cmpd. Group	Compound Math	Signal Type	RT	Type	Scan	Product Ion
prometryn-MS-cut5	2		MS	7.83	Target	MS1 Scan	242.1
prometryn-MS-cut4	2		MS	10.03	Target	MS1 Scan	242.1
prometryn-MS-cut3	2		MS	12.23	Target	MS1 Scan	242.1
prometryn-MS-cut2	2		MS	14.42	Target	MS1 Scan	242.1
prometryn-MS-cut1	2		MS	16.62	Target	MS1 Scan	242.1
prometryn-MS-Sum	2	Response Sum	MS	14.38	Target	MS1 Scan	242.1

Figure 182: Quantifier settings in MH Quant method based on ^{2}D MS signal.

3 Setup other parameters in the quant method.

Similar to usual MH quant method creation, other method parameters such as multilevel calibration concentration, retention time extraction window, MS extraction window etc., also need to be taken care of in the method creation step. See below for some examples.

prometryn-Sum 1 0.0

Calibration **A**

Level	Conc.	Response	Enable
1	0.0020		☑
2	0.0050		☑
3	0.0100		☑
4	0.0200		☑
5	0.1000		☑

Quantifier **B**

Name	Type	RT	Left RT Delta	Right RT Delta	RT Delta Units
prometryn-cut4	Target	9.98	2.000	2.000	Percent
prometryn-cut3	Target	12.13	2.000	2.000	Percent
prometryn-cut2	Target	14.38	2.000	2.000	Percent
prometryn-cut1	Target	16.58	2.000	2.000	Percent
prometryn-Sum	Target	14.38	2.000	2.000	Percent

Quantifier **C**

Name	Type	Extract Left m/z	Product Ion	Extract Right m/z	MZ Extraction Window Units
prometryn-cut4	Target	100.00	242.1	200.00	PPM
prometryn-cut3	Target	100.00	242.1	200.00	PPM
prometryn-cut2	Target	100.00	242.1	200.00	PPM
prometryn-cut1	Target	100.00	242.1	200.00	PPM
prometryn-Sum	Target	100.00	242.1	200.00	PPM

Figure 183: Other quant method parameter settings. (A) Concentration levels (B) RT window (C) MS extraction window

4 Validate and Analyze

Validate the method to make sure no errors or warnings. Save the method and analyze the whole batch.

5 View result

In the result window, summed compound will show as multiple integrated peaks. Examine the individual peaks and adjust integration if needed. Choose and adjust appropriate calibration curve fit.

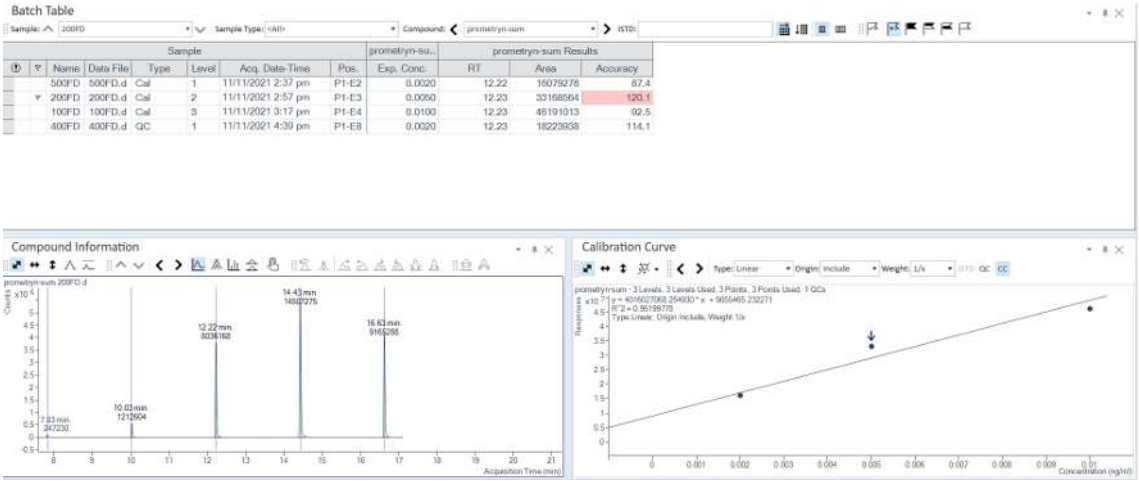


Figure 184: Result window including **Batch Table** (top), **Compound Information** (lower left) and **Calibration Curve** (lower right).

GC Image Basic Information

Typically very complex samples are analyzed by comprehensive 2-dimensional liquid chromatography. The compounds which are often co-eluting form the first dimension are further separated in the second dimension. With the Agilent OpenLab 2D-LC Software always one large data-file spanning the run-time of the two-dimensional analysis will be acquired. As an example, a 2-dimensional analysis of a mixture of 26 polyphenolic standard compounds is shown in a one dimensional data analysis display (Figure 185 on page 287). Especially the heart-cut data can be analyzed with Agilent MassHunter Qualitative and Quantitative Analysis software.

But for easier data analysis and a better visualization of the comprehensive 2D-LC data acquired with the MassHunter Workstation, special software is recommended. Agilent recommends GC Image LCxLC edition Software from GC Image LLC, Nebraska, USA. A trial download can be found on www.GCImage.com as well as an online manual. Agilent 2D-LC data files also including UV spectra and mass spectra data can be directly imported. This software, with the information of the modulation time, is capable to extract the data and isolate each second dimension run. Data will be reconstructed in a two-dimensional display of the retention times. This can be displayed as a colored 2-dimensional map of compound peaks (Figure 186 on page 287). After baseline correction the peaks can be automatically detected by a peak detection algorithm inherent in the 2D-LC data analysis software (Figure 187 on page 288). Since the third dimension is the intensity of the peaks a 3-dimensional plot of the data is possible (Figure 188 on page 288). With the given data set further qualitative and quantitative data analysis is possible.

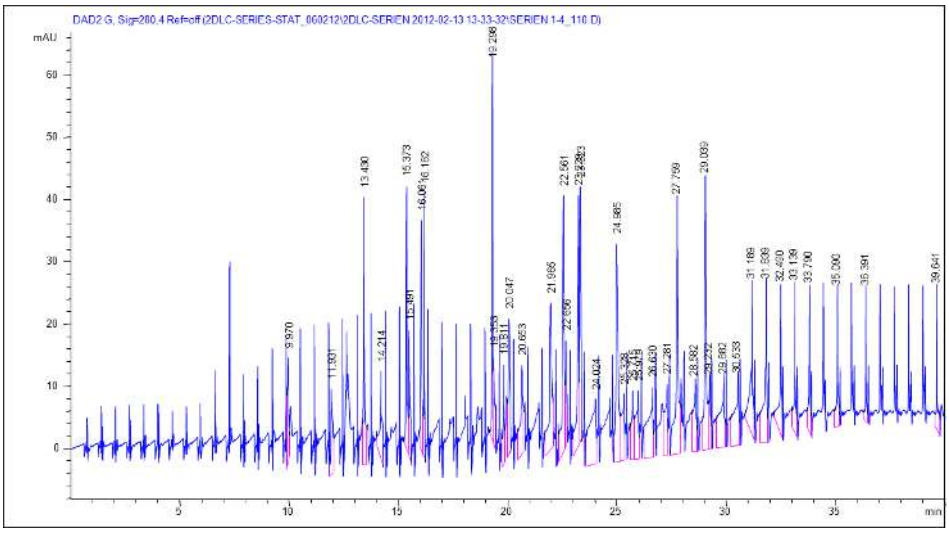


Figure 185: Display of two-dimensional LC data with a one-dimensional data analysis software

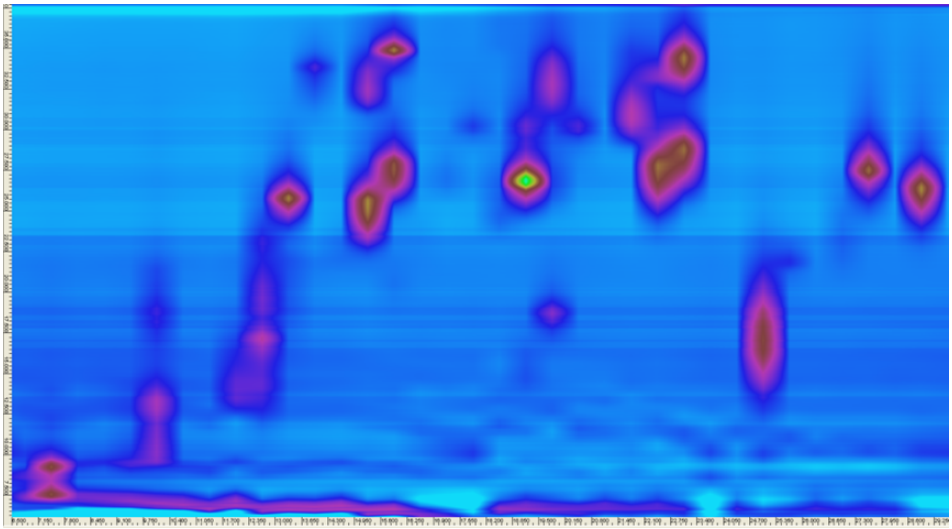


Figure 186: 2D-LC plot of the optimized separation of 26 polyphenolic compounds

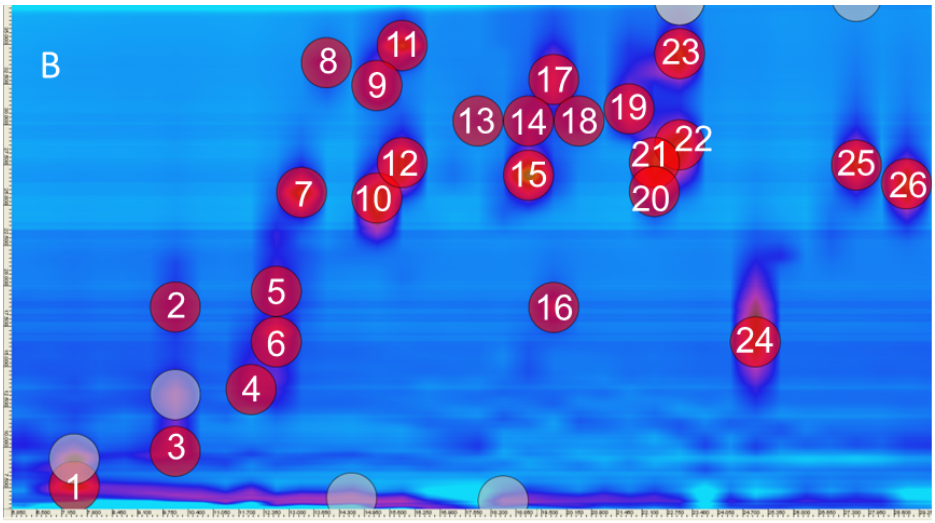


Figure 187: 2DLC plot after baseline correction and with software detected peak annotation

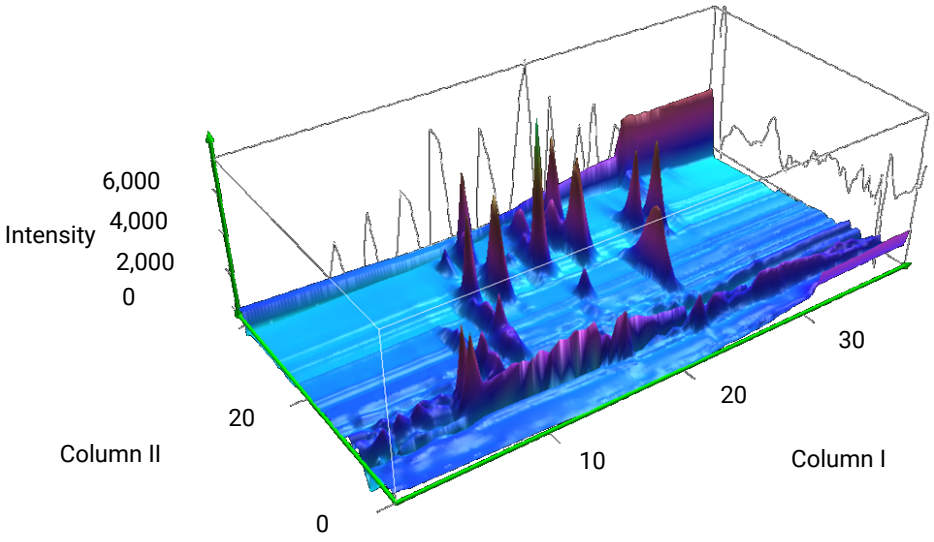


Figure 188: 3-Dimensional display of the separation of the 26 compound standard mixture. The first dimension separation takes 40 minutes and each second dimension separation takes 39 seconds. The back side shows a generated first dimension chromatogram and gives the impression which peaks are coeluting and separated in the second dimensions.

Overview

GC Image LC x LC Edition (short GC Image) is a software for visualization and data analysis of full comprehensive two-dimensional liquid chromatograms:

- M8700AA GC Image LCxLC Edition for UV and Single Quad measurements
- M8710AA GC Image LCxLC-HRMS Edition for UV and/or High-Resolution MS measurements (Q-TOF)

Installation

Parts required

Qty.	p/n	Description
1		Description
1		CD with software
1		License dongle (Wibu Key)
1		Activation code

1. The CD contains two executables: LCxLC2.9-MPr3-64bit.exe (or higher), LCxLC2.9-MPr3-HRMS-64bit.exe (or higher). Choose the appropriate version for your operating system. Corresponding versions are available for the UV only detection.
2. Double-click the chosen executable and follow the instructions on the screen.
3. Activate the software with the USB key. Insert the USB dongle and wait. The driver will install automatically.
4. Activate R2.9 (or higher) in the Windows Start Menu.
5. Enter the activation code, which is shipped with the software.

Use GCImage Software

GCImage is a powerful expert software with many sophisticated features for display, data analysis, compound identification, library search, workflow automation, reporting etc.

The basic knowledges to successfully use the software are the following:

- Import 2D ChemStation data files
- Setting the modulation period
- Choosing a color mapping
- Navigate in the display
- Navigate in the display
- Detect peaks (Blobs)

Preparations

- The USB dongle needs always to be inserted when working with GCImage software. If not, you will be asked to insert it.

Basic knowledges

1 Start up LCImage

LCImage offers optionally a password protected user management system. If you don‘t need it, simply click „Login with system“, which is based on Windows user account.

2 Import the UV signal from the second dimension detector.

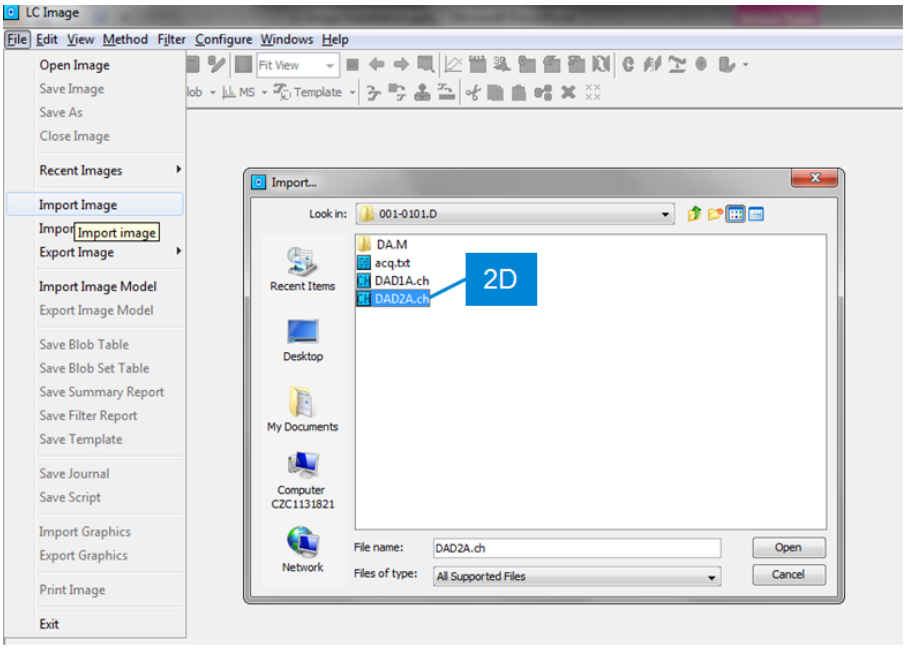


Figure 189: Import UV signal

NOTE

The newer 2D-LC OpenLab or MassHunter file types are not compatible with older GC Image Software versions. So in some cases it is necessary to change the file type, for example to AIA format (**.cdf**), so that you can open it with the available software.

3 Import parameters

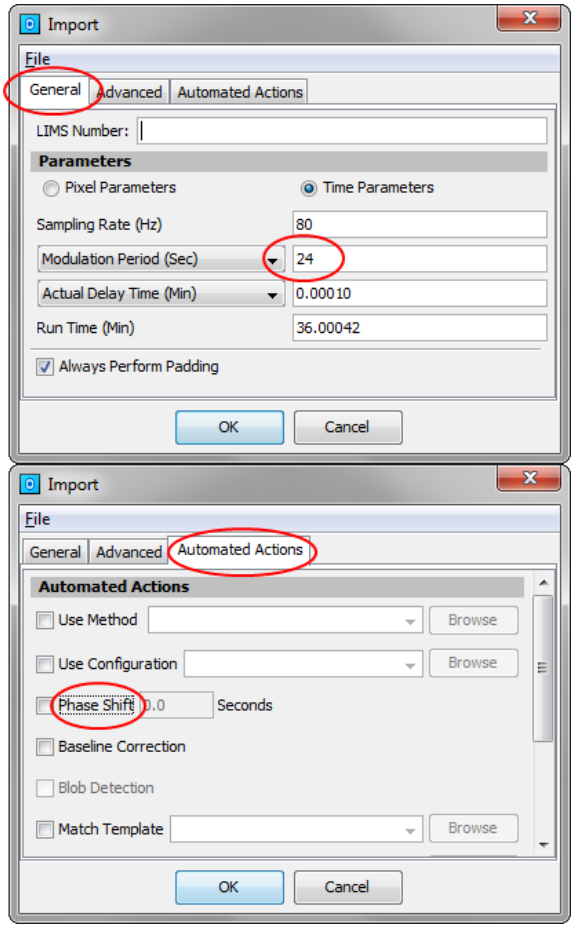
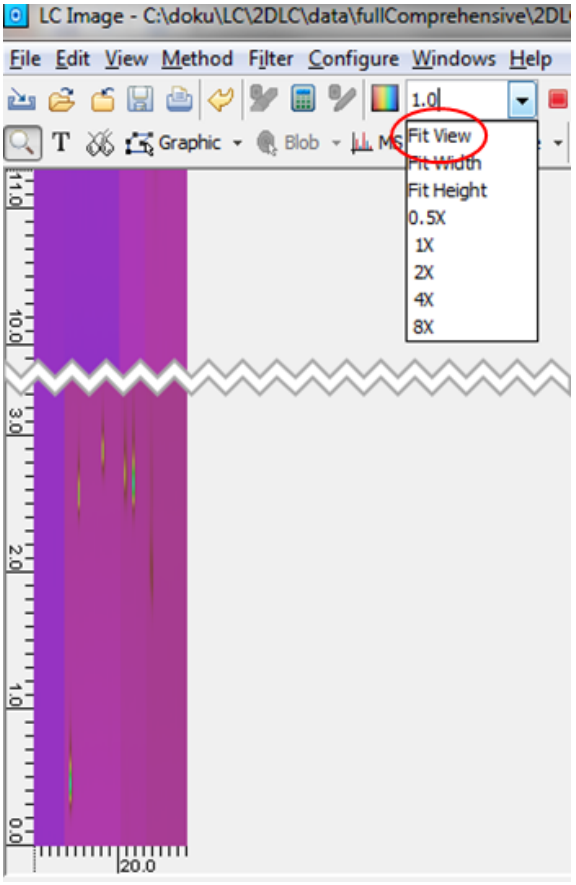


Figure 190: Import parameters

4 Fit view


LC Image - C:\doku\LC\2DLC\data\fullComprehensive\2DL
File Edit View Method Filter Configure Windows Help
1.0
Graphic
Blob
Fit View
Fit Width
Fit Height
0.5X
1X
2X
4X
8X
11.0
10.0
3.0
2.0
1.0
0.0
20.0

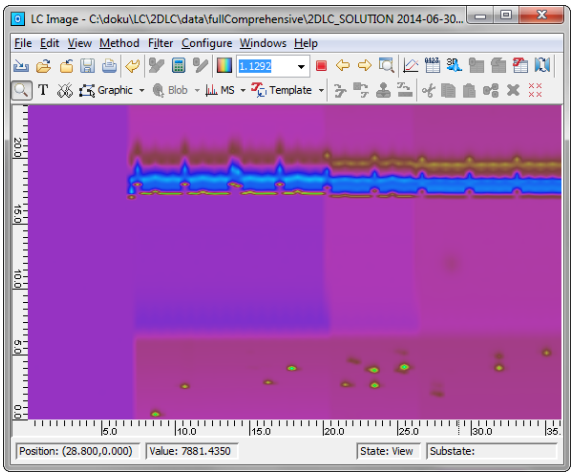


Figure 191: Fit view

5 Correct Baseline

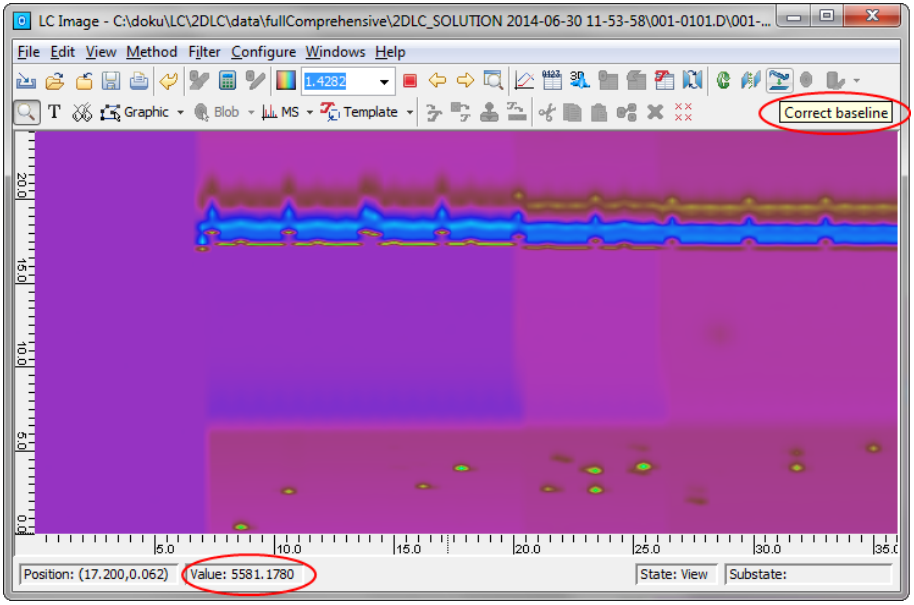


Figure 192: Baseline correction

6 Shift phase

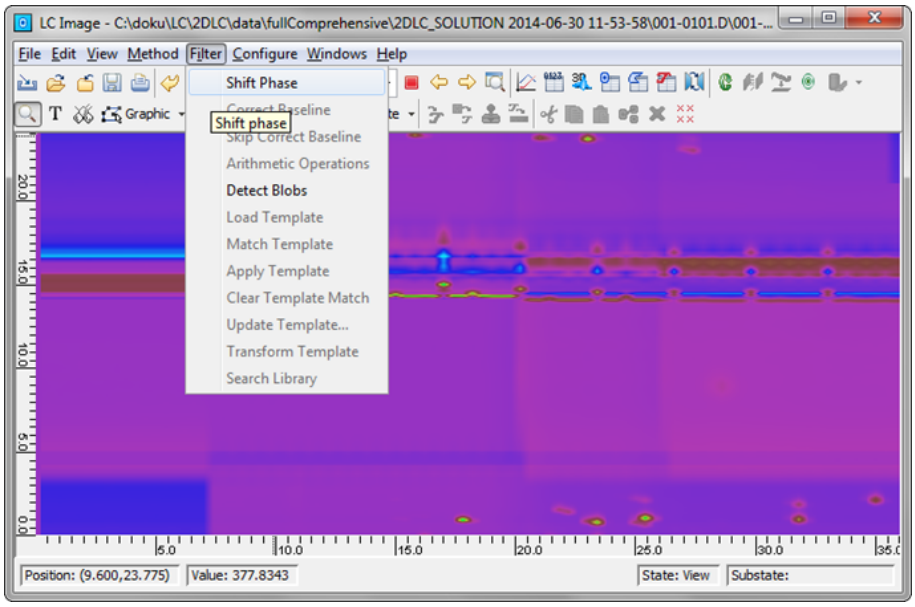


Figure 193: Shift phase

7 Zoom into an interesting region by using the right mouse button and dragging over the display

8 Adjust colors: LC Image offers refined possibilities for optimizing the color scales. Play around with settings for improving the contrast.

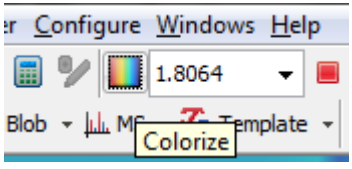


Figure 194: Colorize

9 Select a data range.

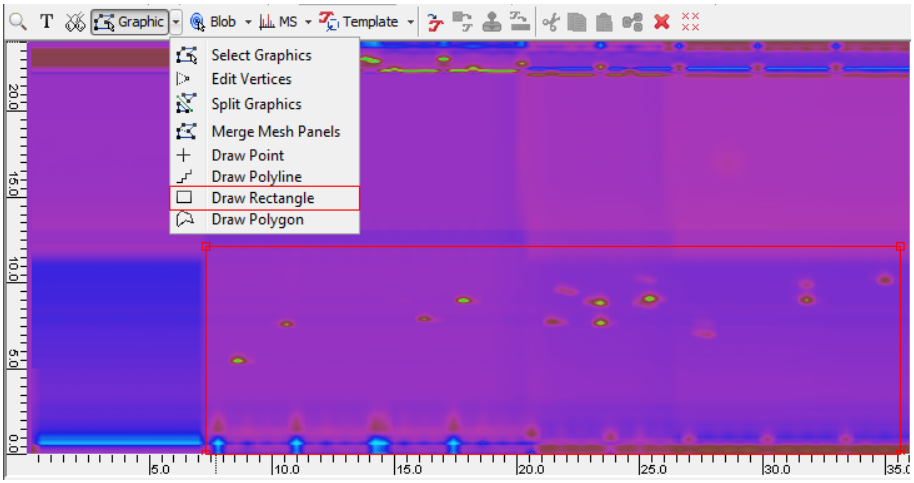


Figure 195: Selection of data range

10 By clicking the „Show 3D perspective“ button or the corresponding menu item, you can easily create a customizable 3D plot.

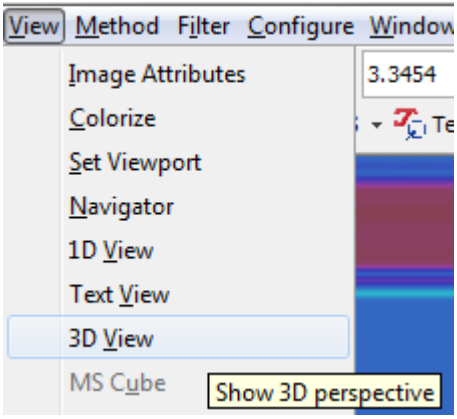


Figure 196: 3D View option

11 View single 2D chromatograms

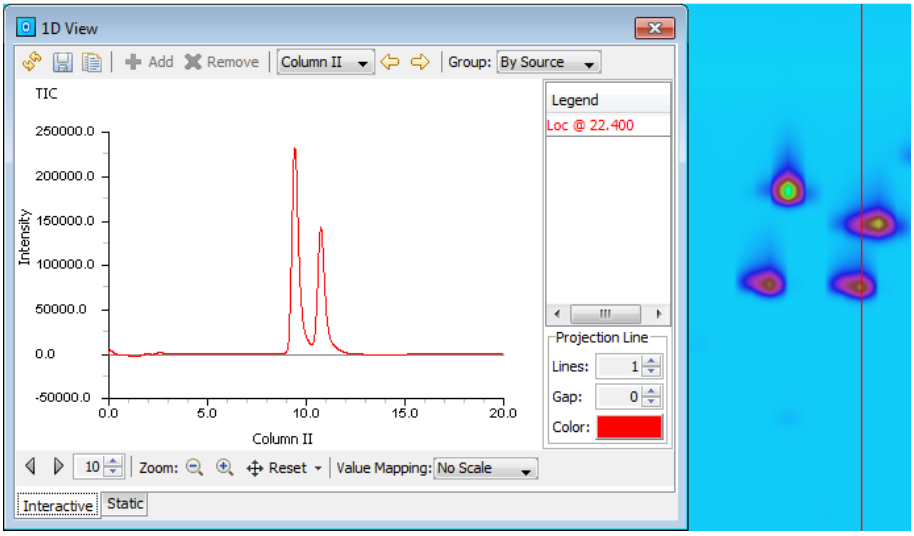


Figure 197: Chromatogram view

12 Select blobs

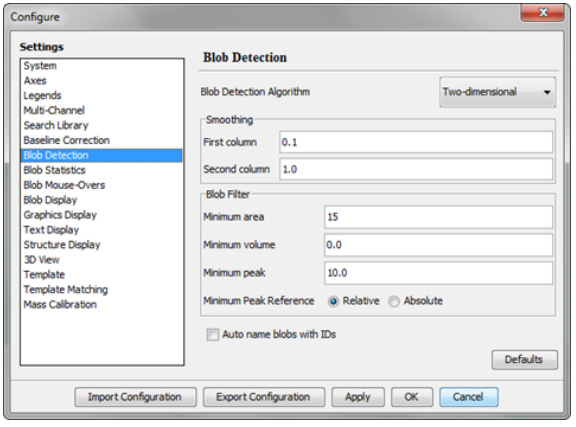


Figure 198: Blob Detection

MS Data

1 Import MS data: The import functionality of MS data is very similar to those of UV measurements. Additionally, you can for example filter to a certain mass range („range limit"), that you are interested in.

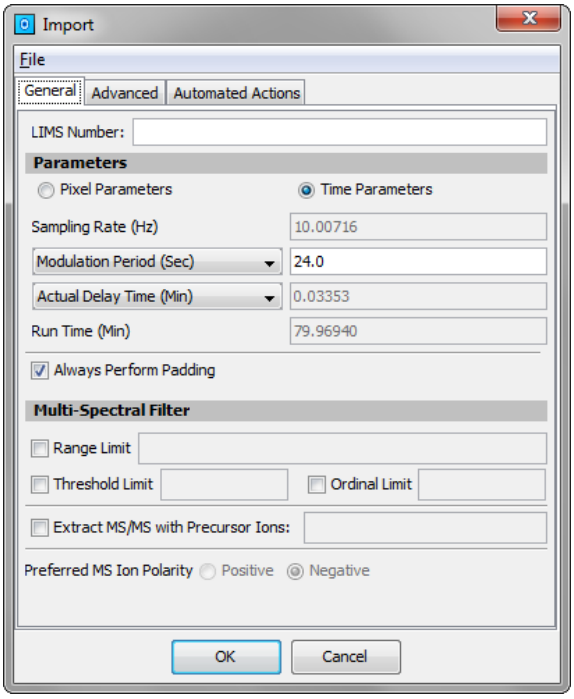


Figure 199: Import of MS data

2 By clicking on „Show 1D view", you can display the TIC for that 2D slice.

3 By clicking on data points or blobs in the 2D view, you can display MS spectra of corresponding plots.

Investigate the effects of using different gradients in ^{2}D

When combining separation systems with related separation mechanisms in the first and second dimension (as in RPxRP), orthogonality is limited. As a result, only a part of the available two-dimensional separation space will be occupied. In such a case, shifted gradients in the second dimension can be used to enlarge the accessible two-dimensional separation space.

1 To investigate the effects of using different gradients in the second dimension, firstly run a comprehensive 2D-LC separation with the same second dimension gradient from 5 – 95 % B repeated during the whole run.

The ^{1}D pump method should be set up as during the checkout runs (see below):

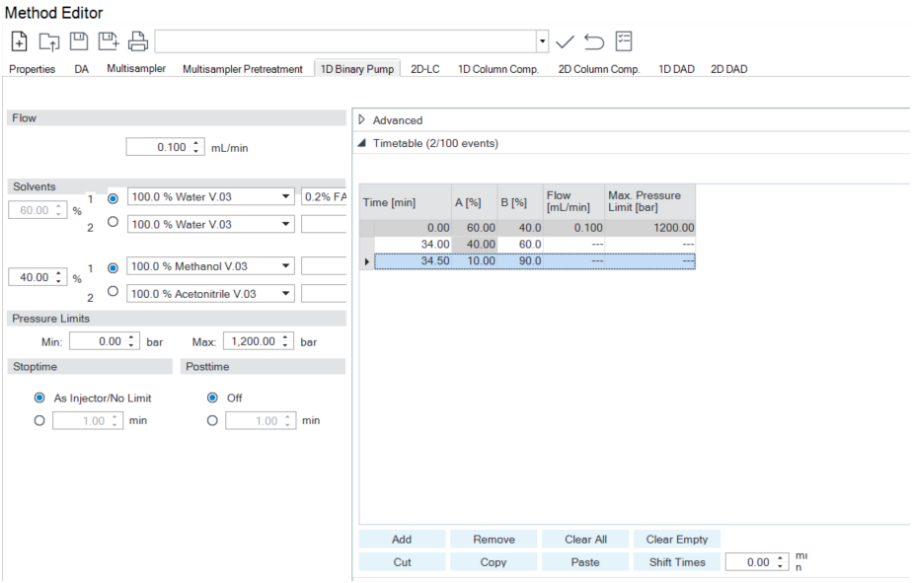


Figure 200: 1D Binary Pump method

2 In 2D-LC System> ^{2}D Pump, set up a ^{2}D pump and modulation method with repeating gradients from 5 – 95 % B as shown below:

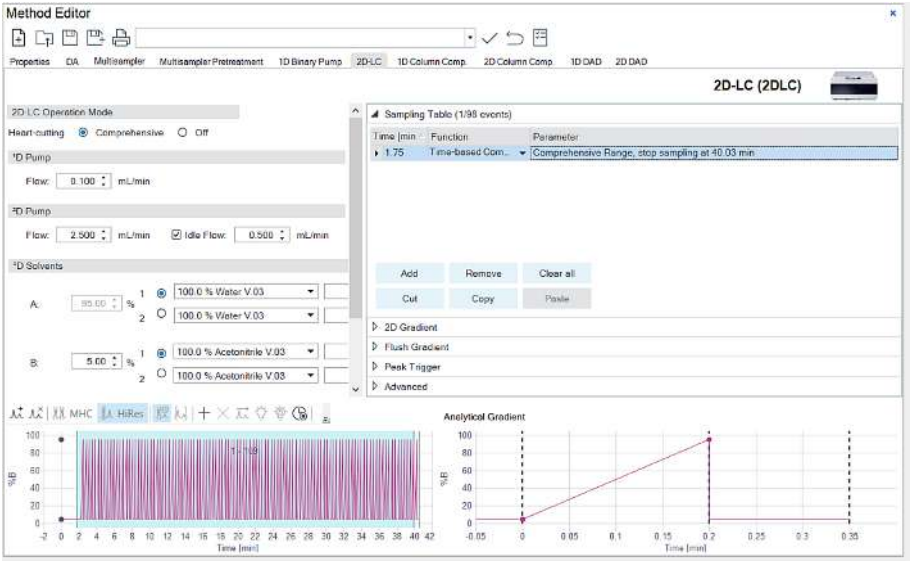


Figure 201: 2D-LC modulation method properties

3 Run the comprehensive 2D-LC analysis.

The resulting separation should look similar to the one shown below:

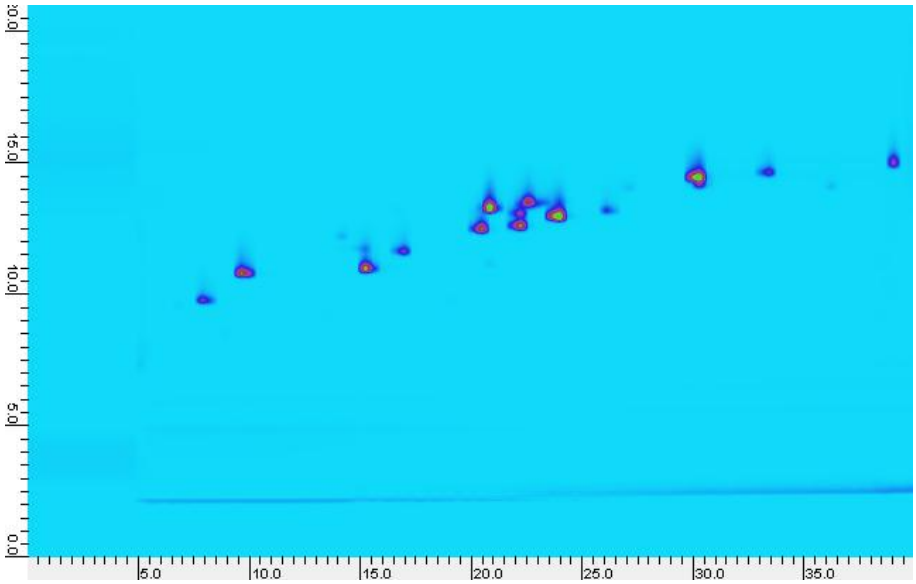


Figure 202: Example of separation after comprehensive 2D-LC analysis

NOTE

Notice how the peaks are distributed around a diagonal line, indicating related separation mechanisms in the first and second dimension.

4 To improve the separation in ^{2}D, a shallower ^{2}D gradient (e.g. from 25 – 75 % B) could be used. The setup of this ^{2}D method is shown below (this is just shown for explanation; you do not need to run this method!):

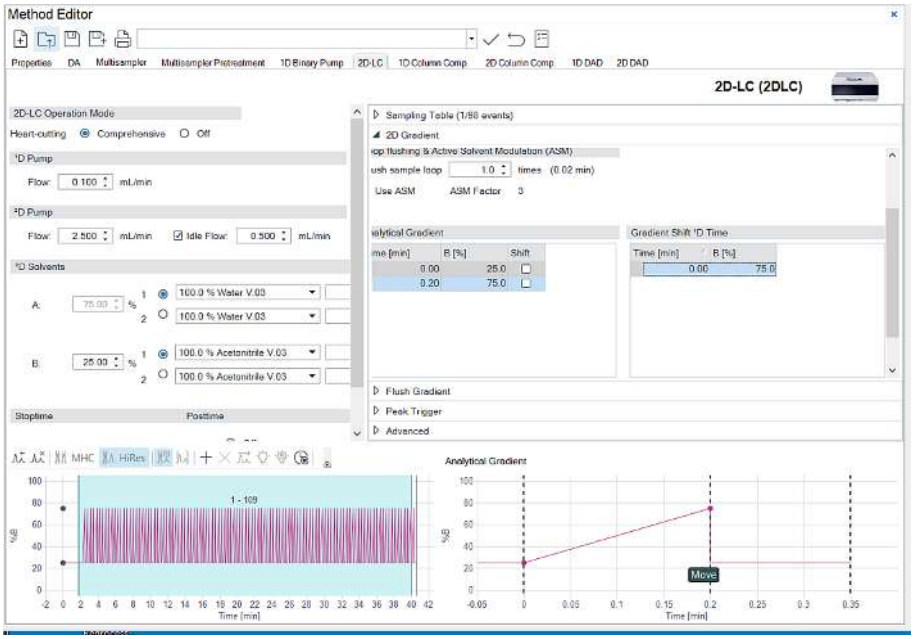


Figure 203: Method setup shallow gradient to improve the ^{2}D separation

The separation resulting from using repeating gradients from 25 – 75 % B in the second dimension is shown below:

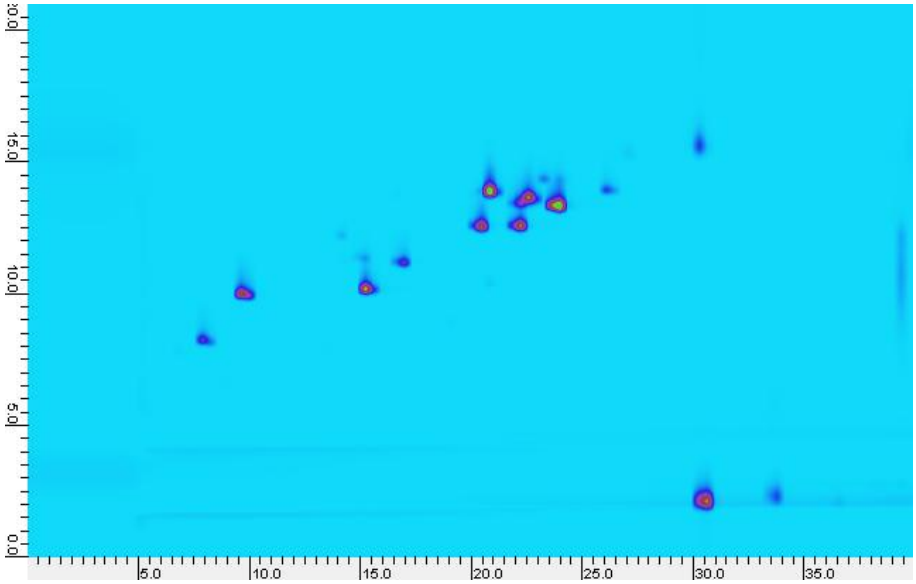


Figure 204: Resulting separation of repeating gradients in second dimension

NOTE

Notice how the peaks are slightly further separated in the second dimension compared to using repeating gradients from 5 – 95 % B. Also notice that the last peaks eluting from the first dimension column are not eluted in one modulation cycle from the second dimension column (wrap-around; see marked area).
To be able to use even shallower gradients in the second dimension to further improve the separation and to also avoid the occurrence of wrap-around, continuously shifted gradients can be used in the second dimension (as was done during the checkout runs).

5 Compare the separations resulting from using the same second dimension gradient (from 5 – 95 % and also from 25 – 75 % B) repeating during the whole run to the separation obtained using continuously shifted second dimension gradients in the checkout run.

NOTE

Notice how the peaks are spread more widely across the two-dimensional separation space (the accessible two-dimensional separation space is enlarged) when shifted gradients are used. Also, notice the effect that using continuously shifted second dimension gradients has on the second dimension retention times of consecutive fractions of the same first dimension peak.

6 Apart from using continuously shifted gradients in the second dimension, as was done during the checkout runs, it is also possible to stepwise shift the second dimension gradients. For this purpose, keep the valve & loop configuration as well as the 1D pump method the same. In **Instrument** > **Setup 2D-LC** , set up a ^{2}D pump and modulation method with stepwise shifted gradients as shown below:

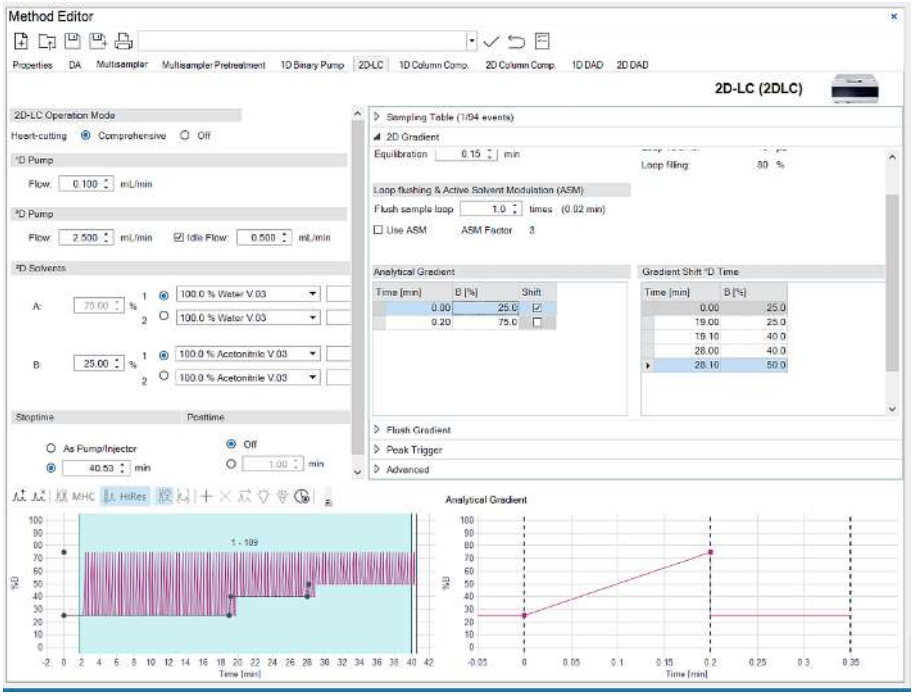


Figure 205: Method of stepwise shifted gradients

7 Run the comprehensive 2D-LC analysis with stepwise shifted gradients in the second dimension.

The resulting separation should look similar to the one shown below:

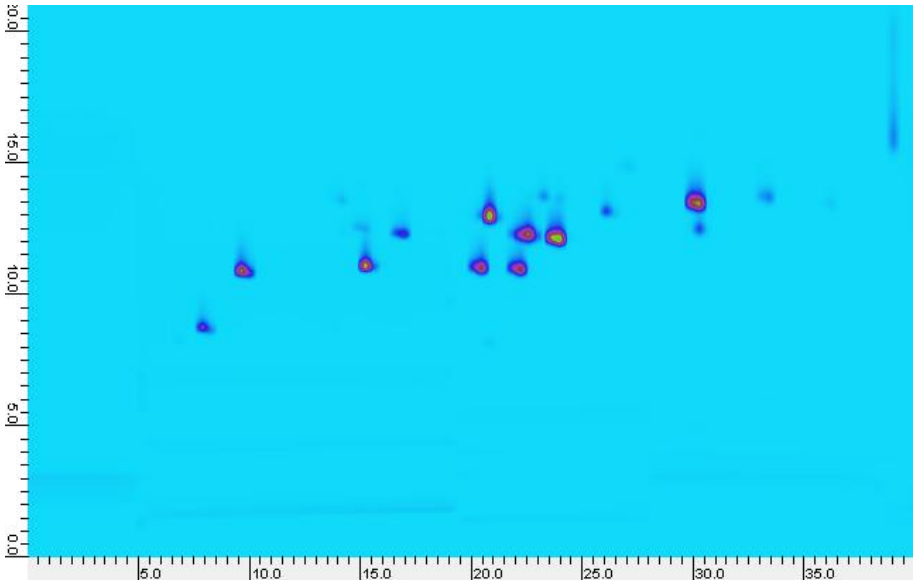


Figure 206: Resulting separation of stepwise shifted gradients in second dimension

NOTE

Notice how consecutive fractions of the same first dimension peak have exactly the same retention time in the second dimension, as they experienced exactly the same second dimension gradient (in contrast to using continuously shifted gradients in the second dimension, which leads to consecutive fractions of one first dimension peak experiencing slightly different second dimension gradients). But be careful! This is only true if the stepwise shifting of the second dimension gradients is performed at times, when no peaks are eluting from the first dimension column.
In case your resulting separation looks different from the one shown above: Your peaks might show a different first dimension retention time due to the use of another first dimension pump (in the separation shown above, a binary pump was used in the first dimension). Check whether the stepwise shifting of the second dimension gradients was performed at times when peaks eluted from the first dimension column in your separation and understand the effect this can have on the second dimension retention times of consecutive fractions of the same first dimension peak!

10 Troubleshooting and Diagnostics

This chapter gives an overview about the troubleshooting and diagnostic features and the different user interfaces.

Overview of the Module's Indicators and Test Functions

For an overview of the module's indicators and test functions, refer to the manuals of the modules installed in your system.

User Interfaces

InfinityLab Assist

InfinityLab Assist provides you with assisted troubleshooting and maintenance at your instrument.

If the system in use supports the InfinityLab Assist, follow the instructions provided. Else, the preferred solution is to use Agilent Lab Advisor Software.

- Depending on the user interface, the available tests and the screens/reports may vary.
- The preferred tool for troubleshooting and diagnostics should be Agilent Lab Advisor Software, see Agilent Lab Advisor Software on page 310.
- Screenshots used within these procedures are based on the Agilent Lab Advisor Software.

Agilent Lab Advisor Software

The Agilent Lab Advisor Software (basic license, shipped with an Agilent LC pump) is a standalone product that can be used with or without a chromatographic data system. Agilent Lab Advisor helps to manage the lab for high-quality chromatographic results by providing a detailed system overview of all connected analytical instruments with instrument status, Early Maintenance Feedback counters (EMF), instrument configuration information, and diagnostic tests. With the push of a button, a detailed diagnostic report can be generated. Upon request, the user can send this report to Agilent for a significantly improved troubleshooting and repair process.

The Agilent Lab Advisor software is available in two versions:

- Lab Advisor Basic
- Lab Advisor Advanced

Lab Advisor Basic is included with every Agilent 1200 Infinity Series and Agilent InfinityLab LC Series instrument.

The Lab Advisor Advanced features can be unlocked by purchasing a license key, and include real-time monitoring of instrument actuals, all various instrument signals, and state machines. In addition, all diagnostic test results, calibration results, and acquired signal data can be uploaded to a shared network folder. The Review Client included in Lab Advisor Advanced makes it possible to load and examine the uploaded data no matter on which instrument it was generated. This makes Data Sharing an ideal tool for internal support groups and users who want to track the instrument history of their analytical systems.

The optional Agilent Maintenance Wizard Add-on provides an easy-to-use, step-by-step multimedia guide for performing preventive maintenance on Agilent 1200 Infinity LC Series instrument.

The tests and diagnostic features that are provided by the Agilent Lab Advisor software may differ from the descriptions in this manual. For details, refer to the Agilent Lab Advisor software help files.

Lab Advisor Instrument Control

Integrated 2D-LC functions in the Lab Advisor Software

This section lists special features, which can be used to get more details and information out of your 2D-LC System. For further details like the diagnostic buffer, the module info, purge pump etc. please check the manuals of each module or the Lab Advisor online help.

NOTE Some of the features are only available if the hardware dongle license for the driver-based 2D-LC solution is installed and active.

2D-LC Hardware License Handling

When

- Installation/Deinstallation of USB Hardware Dongle in the ^{2}D pump of a 2D-LC instrument, to do the following:
- Verify the license status
- Verify the correct installation of the USB dongle
- De-activate the license on the current module, e.g. to transfer the license to a different pump module

Parts required

Qty.	p/n	Description
1		USB Dongle

Software required

- Agilent Lab Advisor Software (2.17 or higher)

Preparations

Read the following

- Documentation provided with the Agilent Lab Advisor online help
- 2D-LC Manual

Preparations

Procedure to follow

- Close the current Acquisition client window
- Close instrument connection from the Control Panel of the CDS

NOTE

The ^{2}D pump must be a 1290 Binary Pump, or any 1290 Infinity II/III High-Speed Pump.

Install the 2D-LC Hardware License

1 Install USB dongle and license, for details, see Licensing the 2D-LC Instrument in MassHunter on page 110.

2 To use the 2D-LC solution, respect that the following can occur:

- The 2D-LC License is active:

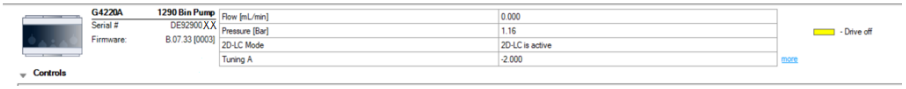


Figure 207: 2D-LC Mode is active

- The hardware dongle is installed
- The ^{2}D pump is configured as a 2D-LC cluster
- The 2D-LC solution if ready for use

- The 2D-LC License is inactive:

Figure 208: 2D-LC Mode is inactive

- The hardware dongle is installed
- The ^{2}D pump recognizes the dongle
- The ^{2}D pump is *NOT* configured in the Chromatography Data System (CDS).

  To use the 2D-LC solution, first configure the 2D-LC cluster, see Configure the 2D-LC Cluster on page 119.

Remove and transfer the 2D-LC license back to the USB dongle

1 Plug in the USB dongle to the ^{2}D pump USB socket.

2 In the Lab Advisor Software, select **Instrument Control** > **2D pump** > **Control section** > **Special command** .

3 Click the **Remove 2D-LC License** button.

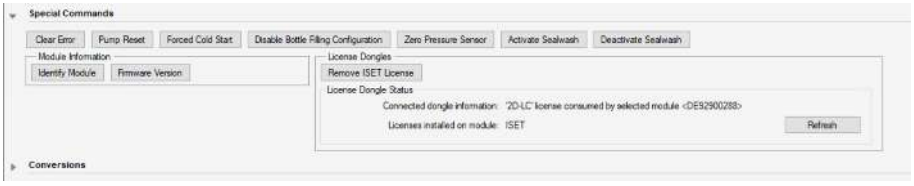


Figure 209: 2D-LC **License Dongle Status** information **2D-LC license is consumed**

This measure has the following consequences:

- The 2D-LC License is transferred back to the USB dongle
- The 2D-LC solution is no longer available on the system

2D-LC Capillaries Configuration Tool

The **Configuration** tool of Agilent Lab Advisor stores by default only standard capillaries. To add 2D-LC specific capillaries (e.g. Sample Loop, transfer capillary, or ASM capillary) to the 2D-LC instrument, it is necessary to configure these capillaries.

When

- Installation of 2D-LC specific capillaries

Parts required

Qty.	p/n	Description
1		All required capillaries for the 2D-LC setup

Software required

- Agilent Lab Advisor Software (2.17 or higher)

Preparations

Read the following

- Documentation provided with the Agilent Lab Advisor online help
- 2D-LC Manual

Take care that all capillaries are installed and their specification is available.

1 In Agilent Lab Advisor, select **Instrument Control** > **2D pump** > **Control** > **Configuration** .

The **Edit Generic Capillaries** function is available.

2 Enter the specific parameters **Length [mm]** and **Diameter [mm]** for the **Generic Sample Loop**, **Generic Transfer Capillary**, and **Generic ASM Capillary** to the fields.

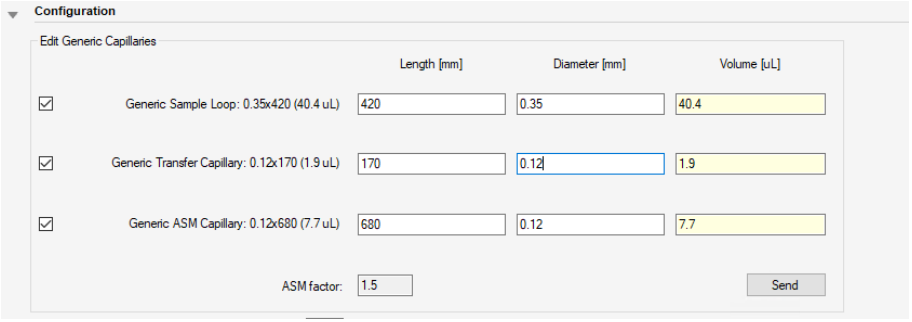


Figure 210: 2D-LC generic capillaries configuration

The Volume [µL] of the specified capillaries is automatically calculated.

3 Click send.

The **Configuration** tool sends the parameters to the 2D-LC system.

The capillaries now appear in the **Modify capillaries** selection list of the chromatographic data system.

Instrument Control of the 2D-LC Cluster

When

- Control the behavior of the ^{2}D pump and the 2D-LC valves.

Software required

- Agilent Lab Advisor Software (2.17 or higher)

Preparations

Read the following

- Documentation provided with the Agilent Lab Advisor online help
- 2D-LC Manual

Preparations

Procedure to follow

- Close the current Acquisition client window
- Close instrument connection from the Control Panel of the CDS

NOTE

To use instrument control of the ^{2}D pump, the 2D-LC hardware license must be active.

1 Select **Instrument Control** of the ^{2}D pump (2D-LC cluster).

2 Change the settings of the ^{2}D pump as required.

3 To identify a valve, select the valve from the **Valve** drop-down list.

The following instrument setups are possible:

- One 2D-LC valve
- Three valves:

- One 2D-LC valve
- Two MHC valves

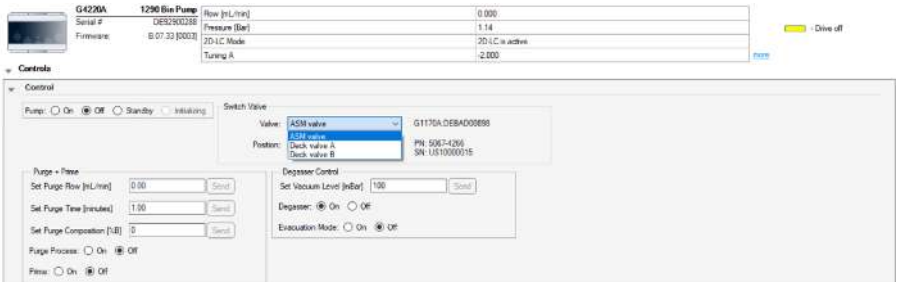


Figure 211: Example of a 2D-LC instrument with a 2D-LC ASM valve and two MHC valves

4 To switch the position of the valve, select the required **Position** from the drop-down list.

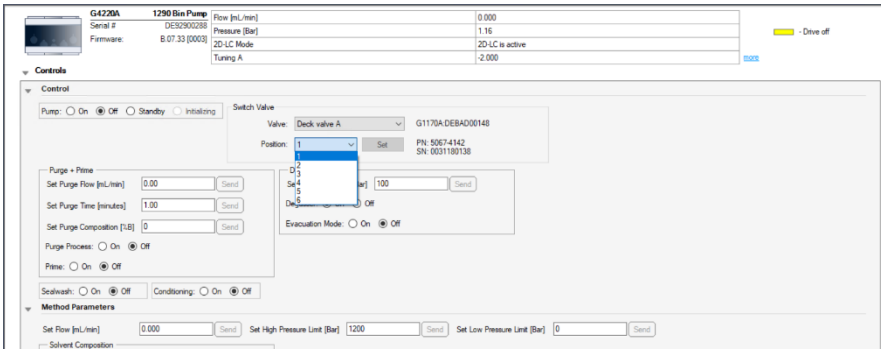


Figure 212: Example of a 2D-LC instrument with a selected MHC valve (Deck A)

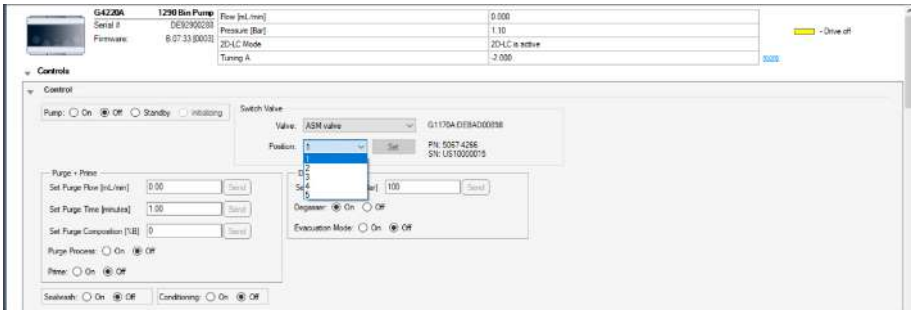


Figure 213: Example of a 2D-LC instrument with a selected ASM valve

Lab Advisor Service & Diagnostic

Decluster the 2D-LC Cluster

This tool allows to remove an LC device's clustering configuration data, e.g. the linking between ^{2}D pump and 2D-LC valve.

When

- Replacement of one of the cluster partners.

Software required

- Agilent Lab Advisor Software (2.17 or higher)

Preparations

Read the following

- Documentation provided with the Agilent Lab Advisor online help
- 2D-LC Manual

Preparations

Procedure to follow

- Close the current Acquisition client window
- Close instrument connection from the Control Panel of the CDS

1 Select **Service & Diagnostic** from the menu.

2 Select the ^{2}D pump.

3 Select **Firmware Declustering**.

4 To **Clear clustering configuration data**, press the **Run** button.

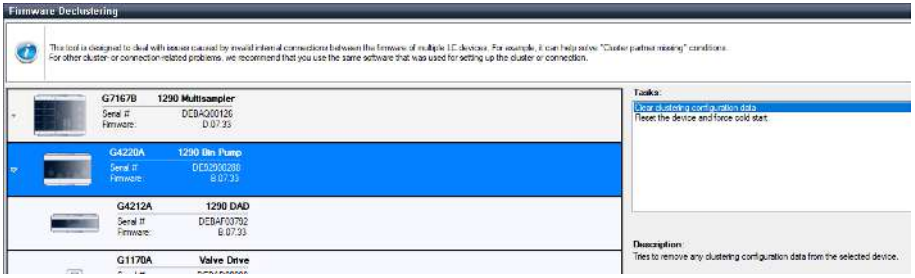


Figure 214: **Firmware Declustering** of the 2D-LC 1290 binary pump

NOTE

To re-establish the link between the two modules, re-perform an Auto configuration and a selection as cluster.

Pump Head Leak Test for the ^{2}D Pump

The test determines the leakage of the individual pump heads.

This 2D-LC test works only for the driver-based 2D-LC solution.

When

- Diagnostic of the ^{2}D pump.

Software required

- Agilent Lab Advisor Software (2.17 or higher)

Preparations

Read the following

- Documentation provided with the Agilent Lab Advisor online help
- 2D-LC Manual

Preparations

Procedure to follow

- Close the current Acquisition client window
- Close instrument connection from the Control Panel of the CDS

1 Select **Service & Diagnostic** from the menu.

2 Select the ^{2}D pump.

3 Select the **Pump Head Leak Test**.

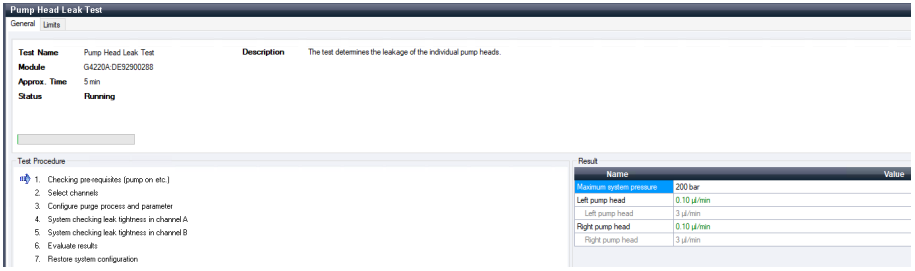


Figure 215: **Pump Head Leak Test** for the 2D-LC 1290 binary pump

4 Press the **Run** button and follow the instructions in the software.

Pump Leak Rate Test for the ^{2}D Pump

The test determines the leak rates in the primary and the secondary pump chambers for component level diagnostic.

This 2D-LC test works only for the driver-based 2D-LC solution.

When

- Diagnostic of the ^{2}D pump.

Software required

- Agilent Lab Advisor Software (2.17 or higher)

Preparations

Read the following

- Documentation provided with the Agilent Lab Advisor online help
- 2D-LC Manual

Preparations

Procedure to follow

- Close the current Acquisition client window
- Close instrument connection from the Control Panel of the CDS

1 Select Service & Diagnostic from the menu.

2 Select the ^{2}D pump.

3 Select the Pump Leak Rate Test.

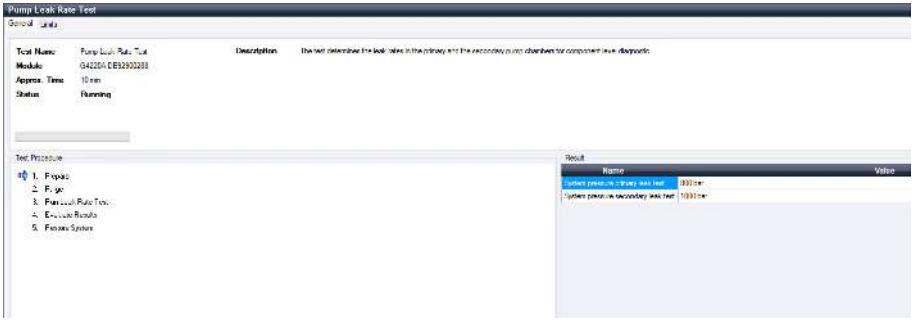


Figure 216: Pump Leak Rate Test for the 2D-LC 1290 binary pump

4 Press the Run button and follow the instructions in the software.

System Pressure Test for the ^{2}D Pump

The test determines the leak tightness of the system between pump and blank nut.

This 2D-LC test works only for the driver-based 2D-LC solution.

When

- Leaks in the system flow path.

Tools required

Qty.	p/n	Description
1		Wrench, 1/4 - 1/5 inch

Parts required

Qty.	p/n	Description
1		Blank nut

Software required

- Agilent Lab Advisor Software (2.17 or higher)

Preparations

Read the following

- Documentation provided with the Agilent Lab Advisor online help
- 2D-LC Manual

Preparations

Procedure to follow

- Close the current Acquisition client window
- Close instrument connection from the Control Panel of the CDS

1 Select Service & Diagnostic from the menu.

2 Select the ^{2}D pump.

3 Select the System Pressure Test.

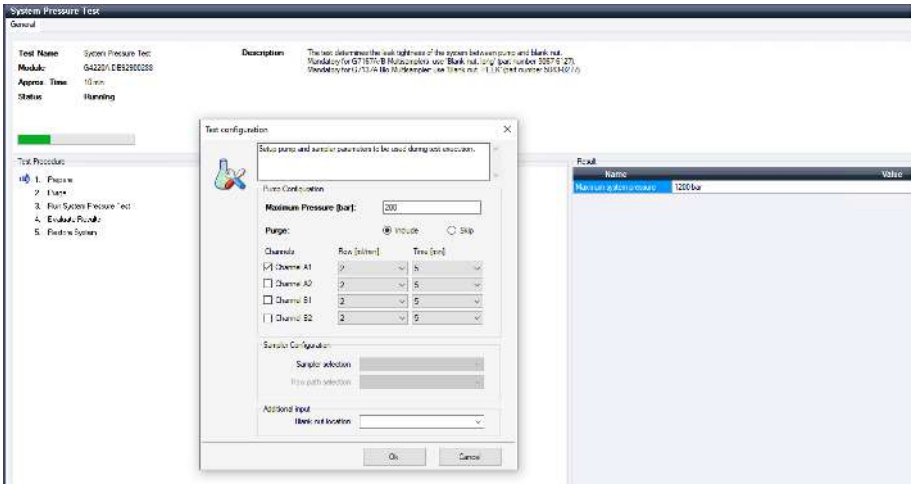


Figure 217: System Pressure Test for the 2D-LC 1290 binary pump

4 Press the Run button and follow the instructions in the software.

2D-LC Capillary Leak Test

Leak and tightness check of the 2D-LC Valve with the ^{2}D pump in the flow path of the second dimension.

This 2D-LC test works only for the driver-based 2D-LC solution.

When

- Leak in the 2D-LC valve.

Tools required

Qty.	p/n	Description
1		Wrench, 1/4 - 1/5 inch

Parts required

Qty.	p/n	Description
1		Blank nut

Software required

- Agilent LabAdvisor Software (2.18 or higher)

Preparations

Read the following

- Documentation provided with the Agilent Lab Advisor online help
- 2D-LC Manual

Preparations

Procedure to follow

- Close the current Acquisition client window
- Close instrument connection from the Control Panel of the CDS

1 Select Service & Diagnostic from the menu.

2 Select the ^{2}D pump.

3 Select the 2D-LC Capillary Leak Test.

4 Press the Run button and follow the instructions in the software.

Replace the Module Firmware

When

Install a newer firmware

- It fixes known problems of older versions, or
- It introduces new features, or
- It ensures keeping all systems at the same (validated) revision

When

Install an older firmware

- It ensures keeping all systems at the same (validated) revision, or
- It ensures compatibility after adding a new module to the system, or
- A third-party control software requires a special version

Tools required

Qty.	p/n	Description
1		Agilent Lab Advisor software

Parts required

Qty.	p/n	Description
1		Firmware, tools and documentation from Agilent web site

Preparations

Read the following

- Documentation provided with the Agilent Lab Advisor online help
- 2D-LC Manual

Preparations

Procedure to follow

- Close the current Acquisition client window
- Close instrument connection from the Control Panel of the CDS

NOTE Do not mix firmware files from different firmware sets.

To upgrade/downgrade the module's firmware carry out the following steps:

1 Download the required module firmware, the latest Lab Advisor software and the documentation from the Agilent web.

http://www.agilent.com/en-us/firmwareDownload?whid=69761

2 For loading the firmware into the module

a Select the folder on the hard drive where the Firmware package is stored.

b Connect the Lab Advisor Software to your 2D-LC instrument.

Figure 218: Firmware Update

c Press the **Lock** button.

The system is locked.

d Select the required firmware version for the Resident and Main Firmware.

e To update the firmware of the instrument, press the **Update** button.

This will require some time.

NOTE Do not interrupt the power supply of the device and the PC during this procedure.

NOTE To avoid problems, select only the firmware file of your connected module and avoid the additional installation of the LC Companion.

The Basic Principle of Troubleshooting

Troubleshooting key Concept – Divide and Conquer

The following troubleshooting concept, shows exemplarily how to approach problems in 2D-LC chromatography.

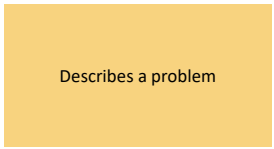
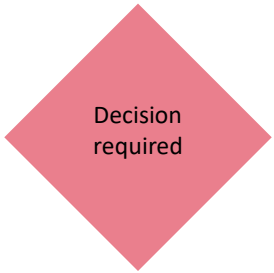
Most of the following explanations can also be used to isolate and detect standard LC issues.

The basic principle of troubleshooting should always be a step by step approach to the 2D-LC problem. As a first step, find out whether the cause of the error is either:

- The application method, or
- The 2D-LC instrument

For a recommended approach to isolate the cause of the issue, see the graphic below. All examples use symbols as described in the following table.

Table 39: Description for symbols as used in troubleshooting decision trees

Symbol	Description
	Shows and describes a problem in the 2D-LC system. Indicates the starting point for a series of actions and decisions leading to a solution for the problem.
	Illustrates, that the user must identify what an observation means. Then the user must take a decision, which further way of troubleshooting to follow.
	Shows, the user must act to proceed and come to the next decision or solution.

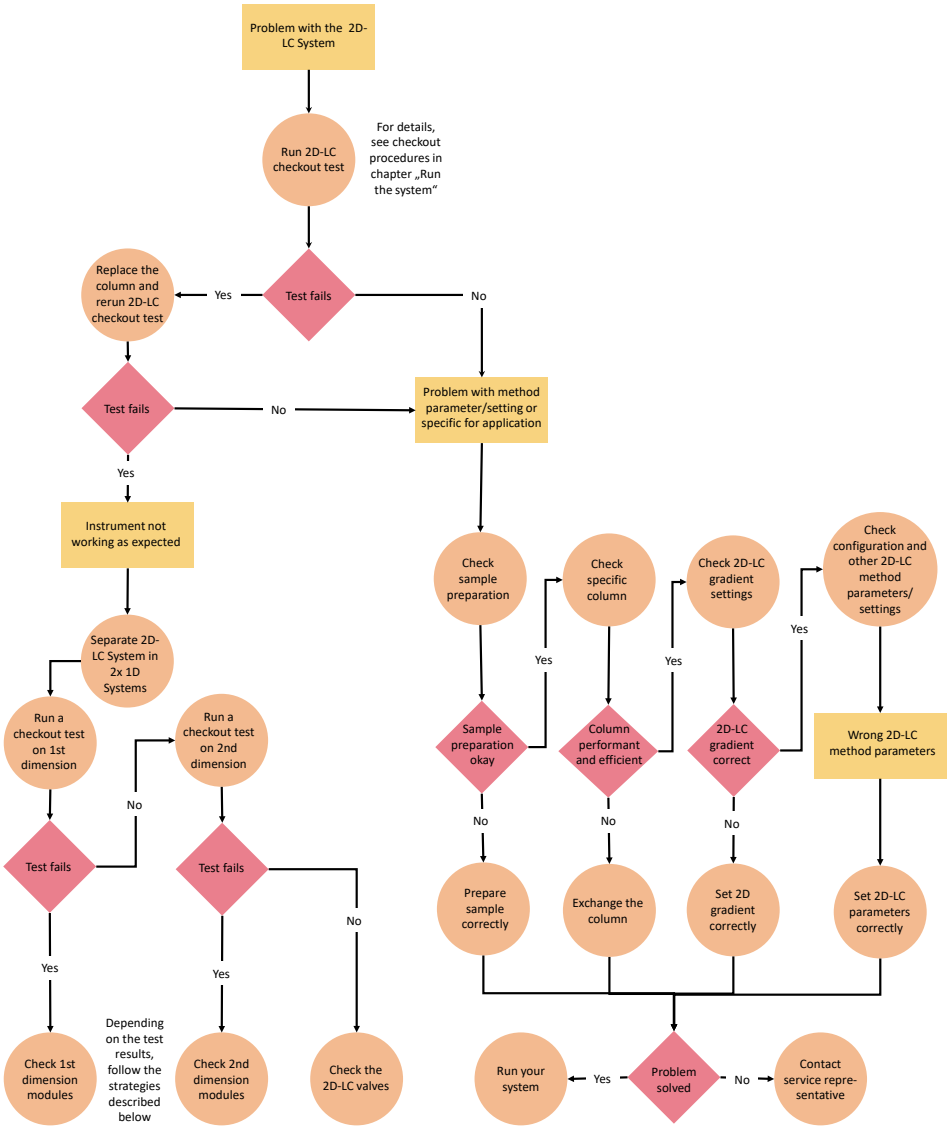


Figure 219: Example for a strategy to identify the application method or instrument as root cause for issues in 2D-LC chromatography

After ruling out the application method as the cause of the issue, one can start to search for the problem's root cause within the 2D-LC Instrument hardware.

Common HPLC hardware issues, along with the location of each problem's respective troubleshooting procedure are listed below:

- Pressure too high on page 331
- Pressure too low on page 332
- Peak area and peak height related on page 333
- Retention time related on page 334
- Missing signal linearity on page 335
- Drifting signal on page 336
- Signal noisy on page 337

Pressure too high

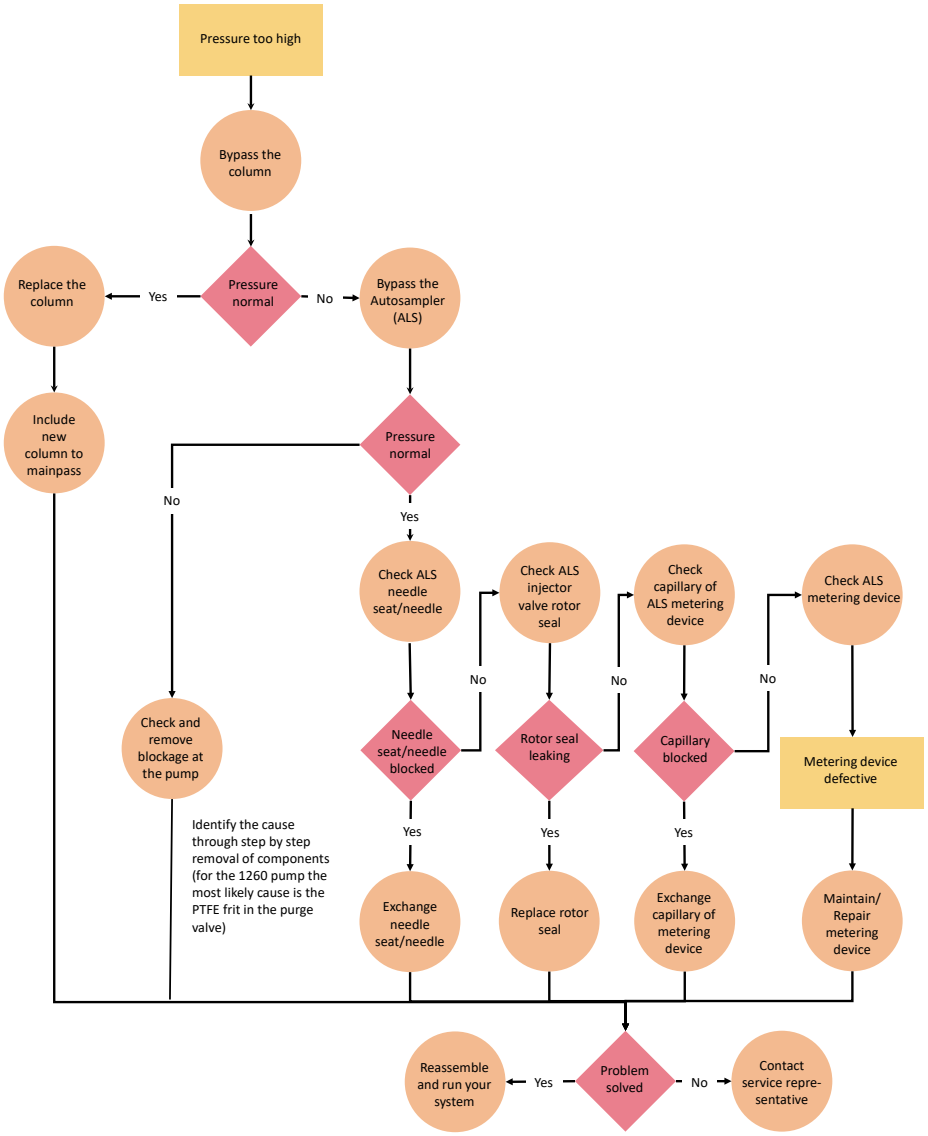


Figure 220: Example for a strategy to eliminate issues related to too high pressure in 2D-LC instruments

Pressure too low

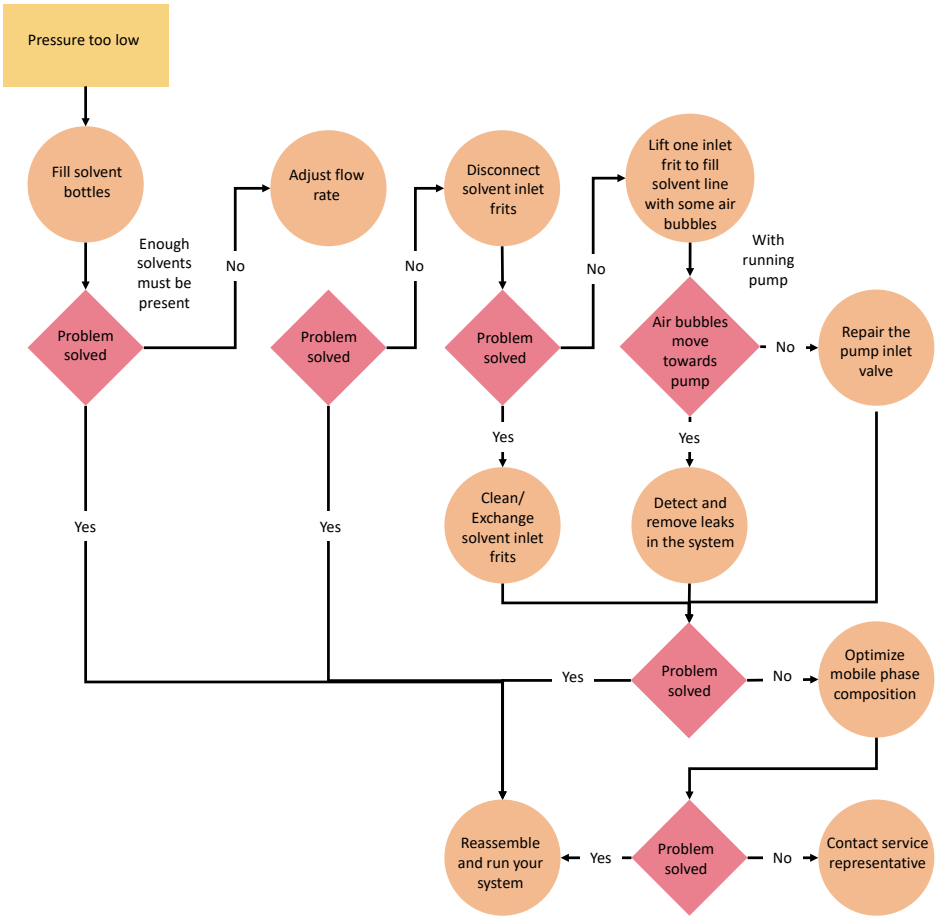


Figure 221: Example for a strategy to eliminate issues related to too low pressure in 2D-LC instruments

Peak area and peak height related

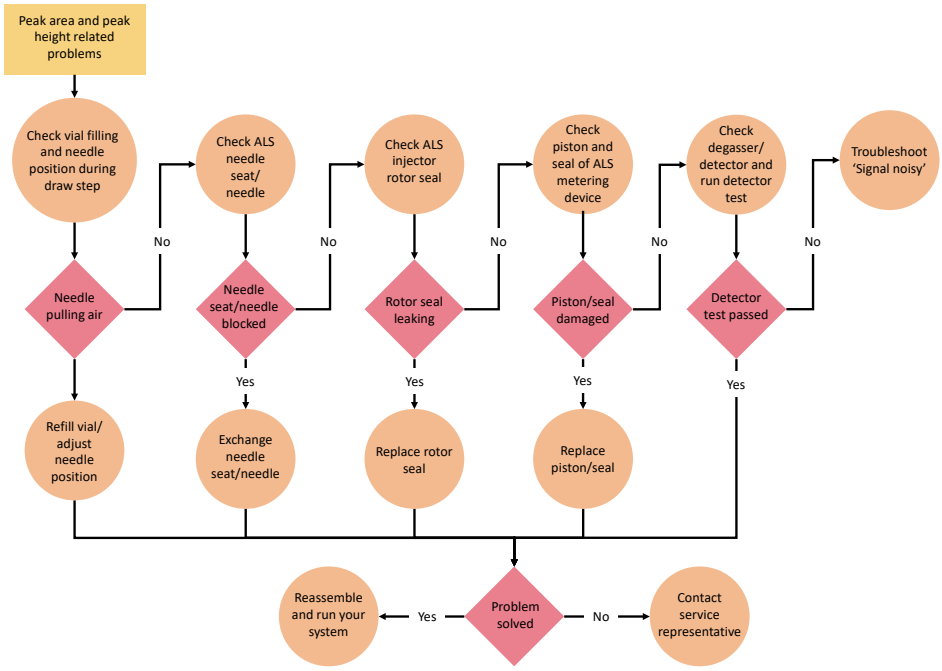


Figure 222: Example for a strategy to eliminate issues related to peak problems in 2D-LC instruments

Retention time related

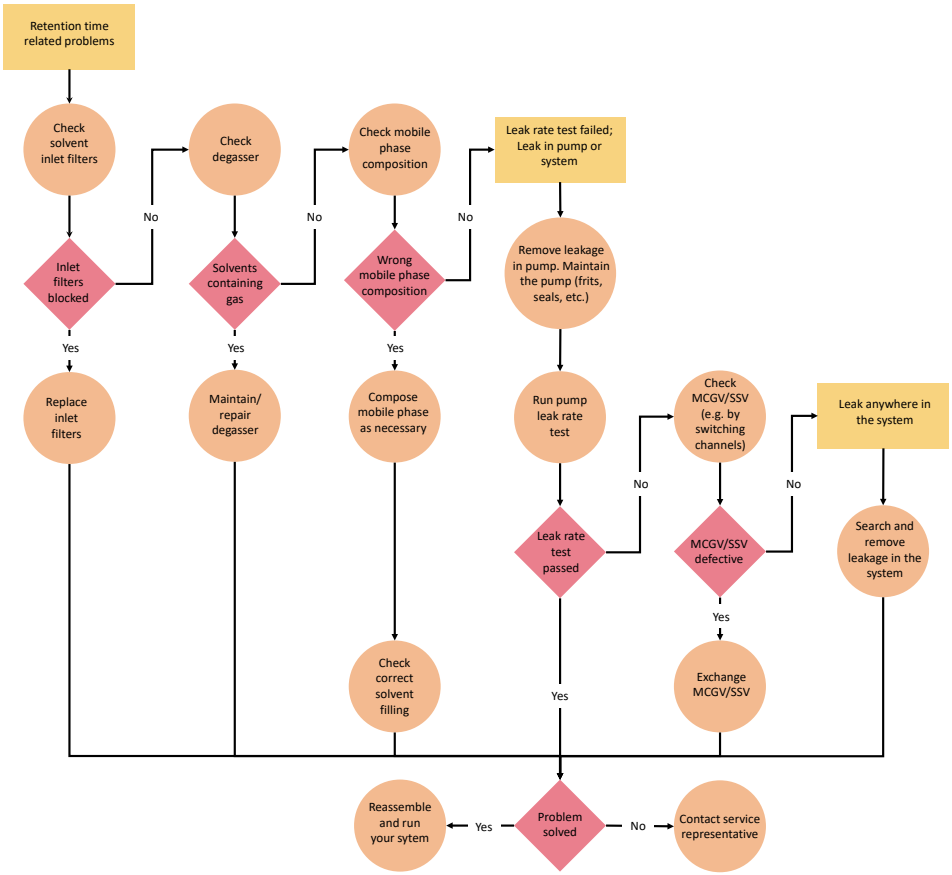


Figure 223: Example for a strategy to eliminate issues related to retention time in 2D-LC instruments

Missing signal linearity

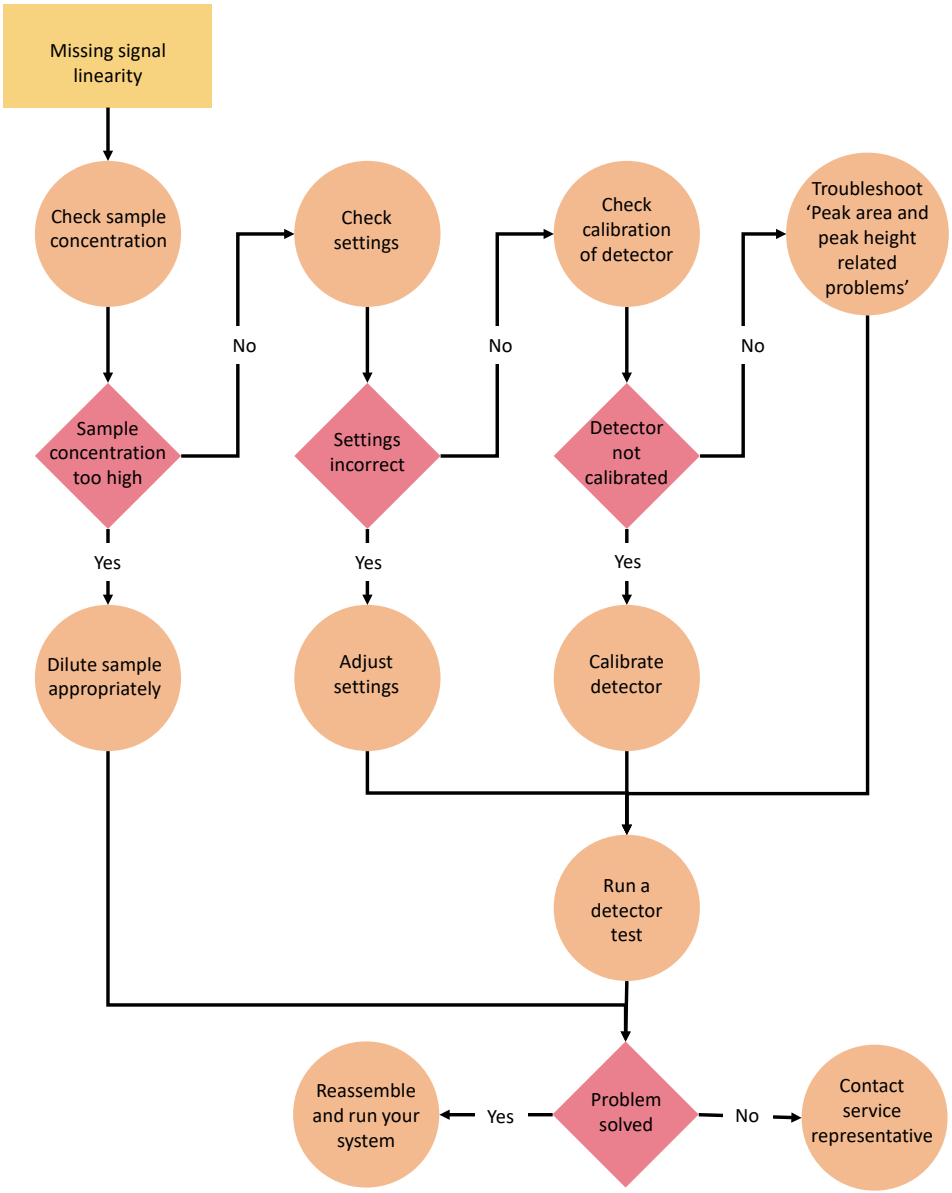


Figure 224: Example for a strategy to eliminate issues related to missing signal linearity in 2D-LC instruments

Drifting signal

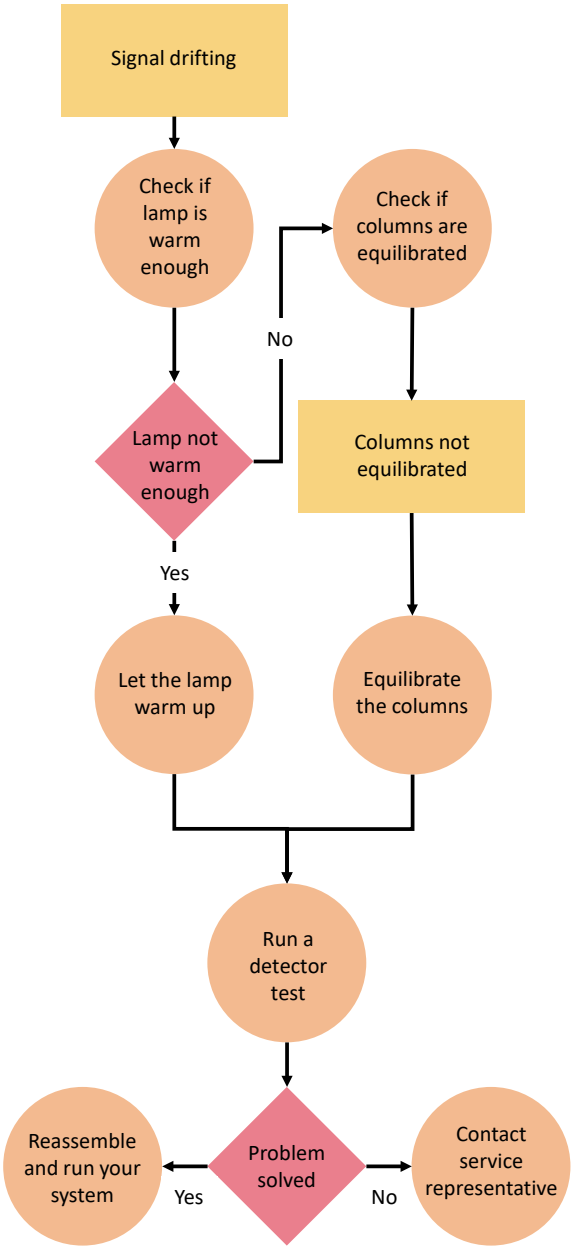


Figure 225: Example for a strategy to eliminate issues related to drifting signal in 2D-LC instruments

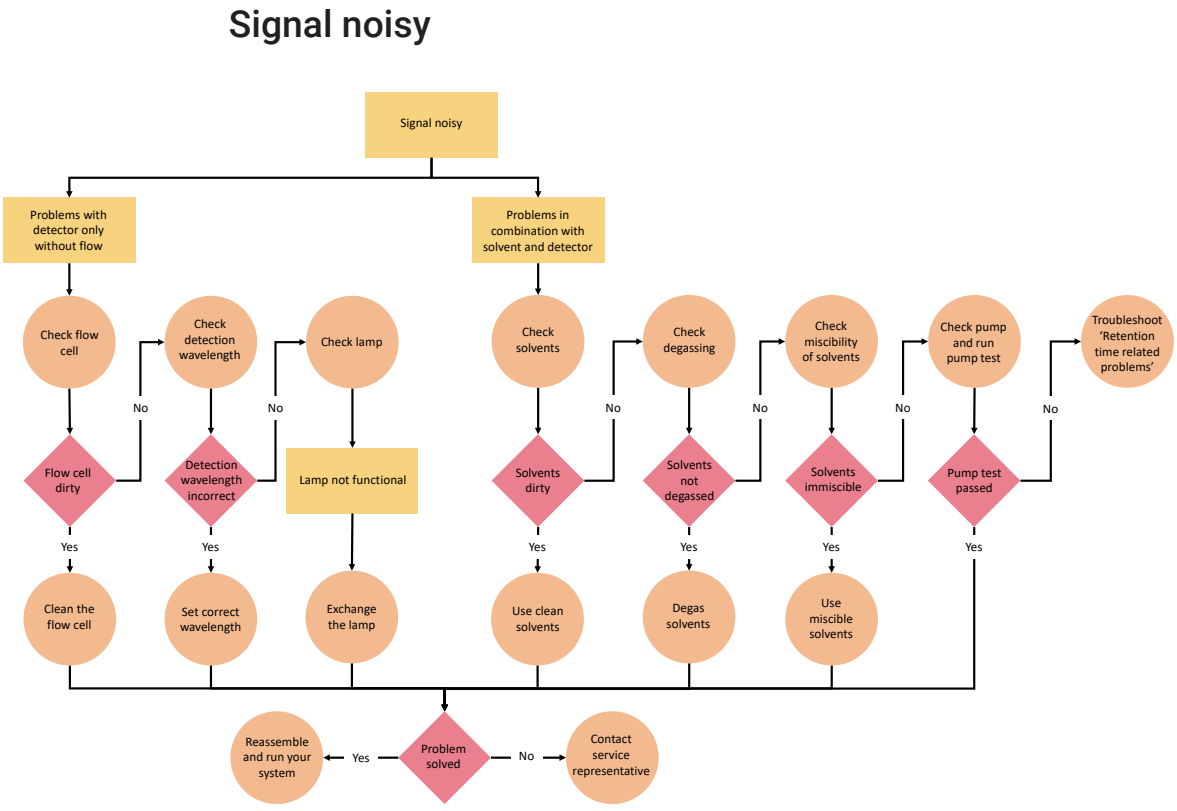


Figure 226: Example for a strategy to eliminate issues related to signal noise in 2D-LC instruments

Recommended Tests to Conclude Troubleshooting

The following table shows the most important tests to conclude troubleshooting.

- For further detailed information, see:
  - Maintenance information in the specific manual of each module.
  - Recommended Tests to Conclude Troubleshooting on page 338
  - *Best Practices for Using an Agilent LC System Technical Note (InfinityLab-BestPractice-en-SD-29000194.pdf, SD-29000194)*
- For additional help, contact your local Agilent Technologies service representative.

Table 40: Recommended Tests for 2D-LC System Troubleshooting

Pump	Column Compartment	Autosampler	Valve	Detector	2D-LC Instrument
Pressure Test Leak Test	Thermostat Test Pressure Test (if column valve is present)	Pressure Test Inject standards or inject different volumes or blanks	Switching valve position/ Check pressure reading Pressure Test Capillary Leak Test (for 2D-LC valve only)	Lamp Intensity Test Wavelength calibration In addition there are detector-specific tests.	Run Checkout For 2D-LC Instruments • Pressure test of the 1D-LC Part • Pressure Test of the 2D-LC Part
Pump characteristic • Pump Ripple (1260 Pump) • Tuning (1290 pump)					

11 Error Information

This chapter describes the meaning of error messages, and provides information on probable causes and suggested actions how to recover from error conditions.

What Are Error Messages

Error messages are displayed in the user interface when an electronic, mechanical, or hydraulic (flow path) failure occurs that requires attention before the analysis can be continued (for example, repair, or exchange of consumables is necessary). In the event of such a failure, the red status indicator at the front of the module is switched on, and an entry is written into the module logbook.

If an error occurs outside a method run, other modules will not be informed about this error. If it occurs within a method run, all connected modules will get a notification, all LEDs get red and the run will be stopped. Depending on the module type, this stop is implemented differently. For example, for a pump, the flow will be stopped for safety reasons. For a detector, the lamp will stay on in order to avoid equilibration time. Depending on the error type, the next run can only be started if the error has been resolved, for example liquid from a leak has been dried. Errors for presumably single time events can be recovered by switching on the system in the user interface.

Special handling is done in case of a leak. As a leak is a potential safety issue and may have occurred at a different module from where it has been observed, a leak always causes a shutdown of all modules, even outside a method run.

In all cases, error propagation is done via the CAN bus or via an APG/ERI remote cable (see documentation for the APG/ERI interface).

If using the InfinityLab Assist, instrument errors will generate a notification. To view the probable causes and recommended actions for this error, click on Help button displayed on the notification.

General Error Messages

General error messages are generic to all Agilent series HPLC modules and may show up on other modules as well.

Timeout

Error ID: 62

The timeout threshold was exceeded.

Probable cause		Suggested actions
1	The analysis was completed successfully, and the timeout function switched off the module as requested.	• Check the logbook for the occurrence and source of a not-ready condition. Restart the analysis where required.
2	A not-ready condition was present during a sequence or multiple-injection run for a period longer than the timeout threshold.	• Check the logbook for the occurrence and source of a not-ready condition. Restart the analysis where required.

Shutdown

Error ID: 63

An external instrument has generated a shutdown signal on the remote line.

Probable cause		Suggested actions
1	Leak detected in an external instrument with a remote connection to the system.	• Fix the leak in the external instrument before restarting the module.
2	Shut-down in an external instrument with a remote connection to the system.	• Check external instruments for a shut-down condition.
3	The degasser failed to generate sufficient vacuum for solvent degassing.	• Check the vacuum degasser for an error condition. Refer to the Service Manual for the degasser or the pump that has the degasser built-in. • Check the external vacuum degasser module (if installed) for an error condition. Refer to the *Service Manual* for the degasser or the pump that has the degasser built-in.

Remote Timeout

Error ID: 70

A not-ready condition is still present on the remote input. When an analysis is started, the system expects all not-ready conditions (for example, a not-ready condition during detector balance) to switch to run conditions within one minute of starting the analysis. If a not-ready condition is still present on the remote line after one minute the error message is generated.

Probable cause		Suggested actions
1	Not-ready condition in one of the instruments connected to the remote line.	• Ensure the instrument showing the not-ready condition is installed correctly, and is set up correctly for analysis.
2	Defective remote cable.	• Exchange the remote cable.
3	Defective components in the instrument showing the not-ready condition.	• Check the instrument for defects (refer to the instrument's documentation).

Lost CAN Partner

Error ID: 71

During an analysis, the internal synchronization or communication between one or more of the modules in the system has failed.

The system processors continually monitor the system configuration. If one or more of the modules is no longer recognized as being connected to the system, the error message is generated.

Probable cause		Suggested actions
1	CAN cable disconnected.	• Ensure all the CAN cables are connected correctly. • Ensure all CAN cables are installed correctly.
2	Defective CAN cable.	• Exchange the CAN cable.
3	Defective mainboard in another module.	• Switch off the system. Restart the system, and determine which module or modules are not recognized by the system.

Leak

Error ID: 64

A leak was detected in the module.

The signals from the two temperature sensors (leak sensor and board-mounted temperature-compensation sensor) are used by the leak algorithm to determine whether a leak is present. When a leak occurs, the leak sensor is cooled by the solvent. This changes the resistance of the leak sensor which is sensed by the leak sensor circuit on the mainboard.

Probable cause		Suggested actions
1	Loose fittings.	• Ensure all fittings are tight.
2	Broken capillary.	• Exchange defective capillaries.

Leak Sensor Open

Error ID: 83

The leak sensor in the module has failed (open circuit).

The current through the leak sensor is dependent on temperature. A leak is detected when solvent cools the leak sensor, causing the leak sensor current to change within defined limits. If the current falls outside the lower limit, the error message is generated.

Probable cause		Suggested actions
1	Defective leak sensor.	• Please contact your Agilent service representative.
2	Leak sensor incorrectly routed, being pinched by a metal component.	• Please contact your Agilent service representative.

Leak Sensor Short

Error ID: 82

The leak sensor in the module has failed (short circuit).

The current through the leak sensor is dependent on temperature. A leak is detected when solvent cools the leak sensor, causing the leak sensor current to change within defined limits. If the current increases above the upper limit, the error message is generated.

Probable cause		Suggested actions
1	Defective leak sensor.	• Please contact your Agilent service representative.
2	Leak sensor incorrectly routed, being pinched by a metal component.	• Please contact your Agilent service representative.

Compensation Sensor Open

Error ID: 81

The ambient-compensation sensor (NTC) on the mainboard in the module has failed (open circuit).

The resistance across the temperature compensation sensor (NTC) on the mainboard is dependent on ambient temperature. The change in resistance is used by the leak circuit to compensate for ambient temperature changes. If the resistance across the sensor increases above the upper limit, the error message is generated.

Probable cause		Suggested actions
1	Defective mainboard.	• Please contact your Agilent service representative.

Compensation Sensor Short

Error ID: 80

The ambient-compensation sensor (NTC) on the mainboard in the module has failed (open circuit).

The resistance across the temperature compensation sensor (NTC) on the mainboard is dependent on ambient temperature. The change in resistance is used by the leak circuit to compensate for ambient temperature changes. If the resistance across the sensor increases above the upper limit, the error message is generated.

Probable cause		Suggested actions
1	Defective mainboard.	• Please contact your Agilent service representative.

Module-Specific Error Messages

For further module-specific errors, please see the manual of the module in question.

Initialization of Valve Failed

Error ID: 24000

During the initialization process the motor of the valve drive moves to some special positions depending on the installed valve head. A failure in this process means either that the movement couldn't be performed properly or it was not noticed correctly by the sensor.

Probable cause		Suggested actions
1	Mechanical problems. Friction too high or blockages on the valve drive's motor or on the valve head.	• Check valve head for correct installation. • Try to identify the source of trouble by installing a different valve head if possible. • Please contact your Agilent service representative.
2	Defect Sensor on the Valve Drive Motor.	• Check valve head for correct installation. • Try to identify the source of trouble by installing a different valve head if possible. • Please contact your Agilent service representative.

Valve Switching Failed

Error ID: 24001

The valve drive was not able to operate the valve head correctly. Either due to mechanical reasons or the movement couldn't be detected correctly.

Probable cause		Suggested actions
1	Mechanical problems. Friction too high or blockages on the valve drive's motor or on the valve head.	• Check valve head for correct installation. • Try to identify the source of trouble by installing a different valve head if possible. • Please contact your Agilent service representative.
2	Defect Sensor on the Valve Drive Motor.	• Check valve head for correct installation. • Try to identify the source of trouble by installing a different valve head if possible. • Please contact your Agilent service representative.

Valve Tag Violation

Error ID: 24006

The valve drive identified a different valve head than it had identified during the last initialization.

NOTE

Soft power-down power supply of the valve drive.
Whenever you want to power cycle the valve drive for a re-boot, it needs to be powered off for at least 10 seconds.

Probable cause		Suggested actions
1	A valve head has been exchanged (hot-plugged) while the valve drive was still powered on.	• Change the valve head. It is important to have the valve switched off for at least 10 s after or before a new valve head has been installed.

Pressure Cluster Partner Missing

Error ID: 2523

The connection from the valve drive to a defined pressure cluster partner is lost.

Probable cause		Suggested actions
1	Communication issues.	• Check the CAN cable connections of the modules.
2	Configuration mismatch.	• Check and correct if necessary the valve configuration and presence of defined pressure cluster partner.

Position Cluster Partner Missing

Error ID: 4526

Probable cause		Suggested actions
1	Communication issues.	• Check the CAN cable connections of the modules.
2	Configuration mismatch.	• Check and correct if necessary the valve configuration and presence of defined position cluster partner. • If the module was moved to another LC stack, perform Firmware Declustering in Service & Diagnostic section of Lab Advisor.

External Valve falls into resident mode

Error ID: Flashing status indicator

The valve drive was not able to operate correctly

Probable cause		Suggested actions
1	Communication issues	• Check the CAN cable connections of the modules. • Check if the hosted module is present.
2	Configuration mismatch	• Check if the firmware on the entire stack is out of the same firmware set. • Check if the limit of 3 hosted modules for each host module is not exceeded. • Check if the dipswitch settings are correct. • Check if the firmware on the entire stack has to be the latest version.

12 Maintenance

This chapter describes the maintenance of the 2D-LC Solution.

Introduction to Maintenance

The 2D-LC solution is designed for easy maintenance. The most frequent maintenance can be done from the front with the modules in place in the system stack. Examples are maintenance of the needle, needle seats, rotor seals, valve heads, or replacing heat exchangers.

Safety Information Related to Maintenance

WARNING Fire and damage to the module

Wrong fuses

- Make sure that only fuses with the required rated current and of the specified type (super-fast, fast, time delay etc) are used for replacement.
- The use of repaired fuses and the short-circuiting of fuse-holders must be avoided.

WARNING Personal injury or damage to the product

Agilent is not responsible for any damages caused, in whole or in part, by improper use of the products, unauthorized alterations, adjustments or modifications to the products, failure to comply with procedures in Agilent product user guides, or use of the products in violation of applicable laws, rules or regulations.

- Use your Agilent products only in the manner described in the Agilent product user guides.

WARNING Electrical shock

Repair work at the module can lead to personal injuries, e.g. shock hazard, when the cover is opened.

- Do not remove the cover of the module.
- Only certified persons are authorized to carry out repairs inside the module.

WARNING Sharp metal edges

Sharp-edged parts of the equipment may cause injuries.

- To prevent personal injury, be careful when getting in contact with sharp metal areas.

WARNING Hot heat exchangers

The column compartment has two heat exchanger assemblies that might be hot.

— **Allow them to cool down before starting repairs.**

WARNING Toxic, flammable and hazardous solvents, samples and reagents

The handling of solvents, samples and reagents can hold health and safety risks.

— **When working with these substances observe appropriate safety procedures (for example by wearing goggles, safety gloves and protective clothing) as described in the material handling and safety data sheet supplied by the vendor, and follow good laboratory practice.**

— **The volume of substances should be reduced to the minimum required for the analysis.**

— **Do not operate the instrument in an explosive atmosphere.**

CAUTION Safety standards for external equipment

— If you connect external equipment to the instrument, make sure that you only use accessory units tested and approved according to the safety standards appropriate for the type of external equipment.

Overview of Maintenance

The following pages describe maintenance procedures (simple repairs) that can be done without opening the main cover.

Table 41: Maintenance procedures

Procedure	Typical Frequency	Notes
Cleaning the Module	If required	
Correcting Leaks	If a leak has occured	Check for leaks
Maintain the Column Switching Valve	If valve leaks	
Replace Valve Heads	If the valve performance shows indication of leakage or wear	
Replacing Parts of the Valve Head	If leak sensor is defective	
Replacing the Fuses of the Infinity Valve Drive	When a fuse is defective	
Replace the Module Firmware	If required	

Cleaning the Module

To keep the module case clean, use a soft cloth slightly dampened with water, or a solution of water and mild detergent. Avoid using organic solvents for cleaning purposes. They can cause damage to plastic parts.

WARNING **Liquid dripping into the electronic compartment of your module can cause shock hazard and damage the module**

- **Do not use an excessively damp cloth during cleaning.**
- **Drain all solvent lines before opening any connections in the flow path.**

NOTE A solution of 70 % isopropanol and 30 % water might be used if the surface of the module needs to be disinfected.

Correcting Leaks

Correcting Leaks (G7116B)

When

- If a leakage has occurred at the heat exchanger or at the capillary connections or at the column switching valve.

Tools required

Qty.	p/n	Description
1		Tissue
1		Pipette
1		Wrench, 1/4 – 5/16 (for capillary connections)

1 Remove the door.

2 Use a pipette and tissue to dry the leak sensor area.

3 Observe the capillary connections and the column switching valve for leaks and correct, if required.

4 Reinstall the door.

Correcting Leaks (G1170A)

When

- If leakage has occured at the capillary connections or at the valve.

Tools required

Qty.	p/n	Description
1		Tissue
1		Pipette
1		Wrench, 1/4 – 5/16 (for capillary connections)

1 Use a pipette and tissue to dry the leak sensor area.

2 Observe the capillary connections and the valve for leaks and correct, if required.

Replace Valve Heads

Replace Valve Heads (G7116B)

Several optional valve heads are available, which can be installed and exchanged easily.

Parts required	Qty.	p/n	Description
	1		Agilent Quick Change Valve Head

WARNING

Toxic, flammable and hazardous solvents, samples and reagents

The handling of solvents, samples and reagents can hold health and safety risks.

- **Be sure that no solvent can drop out of the solvent connections when removing them from your valve head.**
- **When working with these substances observe appropriate safety procedures (for example by wearing goggles, safety gloves and protective clothing) as described in the material handling and safety data sheet supplied by the vendor, and follow good laboratory practice.**

CAUTION

Valve Damage

Using a low pressure valve on the high pressure side can damage the valve.

- When using multiple column compartments as part of a method development solution, make sure that the high pressure valve head is connected to the autosampler and the low pressure valve head is connected to the detector.

NOTE

For details, please refer to the *InfinityLab LC Method Development Solutions User Guide (InfinityLab-Method-Development-Solution-UseMa-en-SD-29000211.pdf, SD-29000211)*.

CAUTION

Column Damage or Bias Measurement Results

Switching the valve to a wrong position can damage the column or bias measurement results.

— Fit the lobe to the groove to make sure the valve is switched to the correct position.

CAUTION

The valve actuator contains sensitive optical parts, which need to be protected from dust and other pollution. Pollution of these parts can impair the accurate selection of valve ports and therefore bias measurement results.

— Always install a valve head for operation and storage. For protecting the actuator, a dummy valve head (part of G1316-67001 (Transportation Lock Kit)) can be used instead of a functional valve. Do not touch parts inside the actuator.

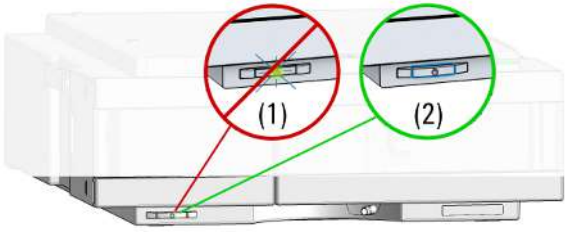
NOTE

The tag reader reads the valve head properties from the valve head RFID tag during initialization of the module. Valve properties will not be updated, if the valve head is replaced while the module is on. Selection of valve port positions can fail, if the instrument does not know the properties of the installed valve.

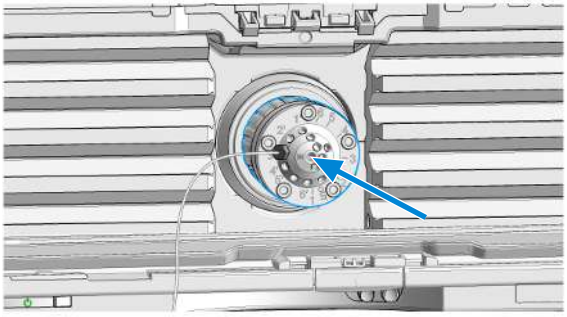
NOTE

To have the valve correctly recognized by the Agilent Infinity Valve Drive you must have the valve drive powered off for at least 10 seconds.

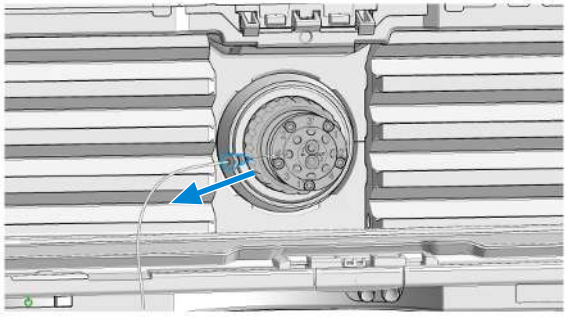
1 Switch off the module.



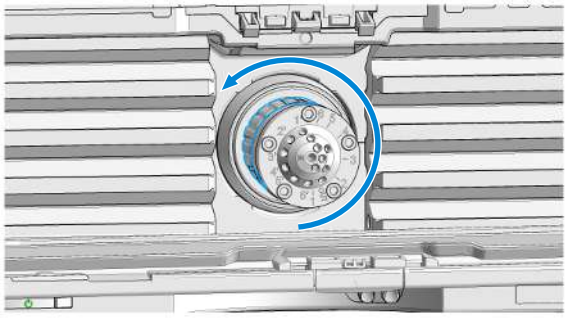
2 Push the valve head to bring it to its outer position.



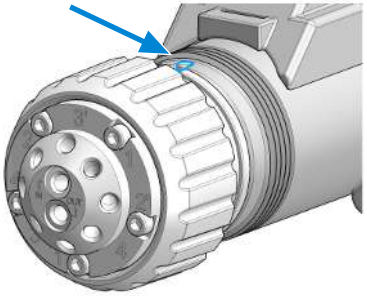
3 Remove all capillary connections from the valve head.



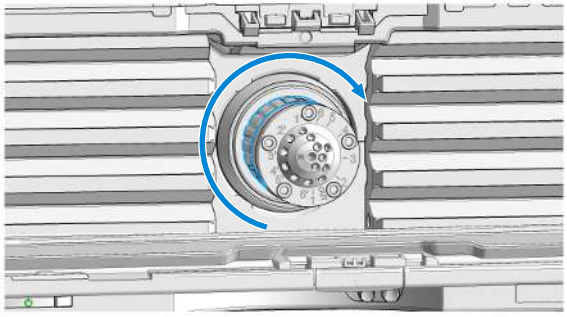
4 Unscrew the nut and remove the valve head.



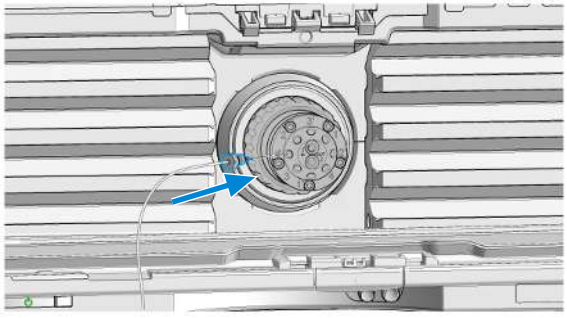
5 Put the new valve head onto the valve drive such that the lobe fits to the groove (see also G7116B_Installation of the Valve Heads).



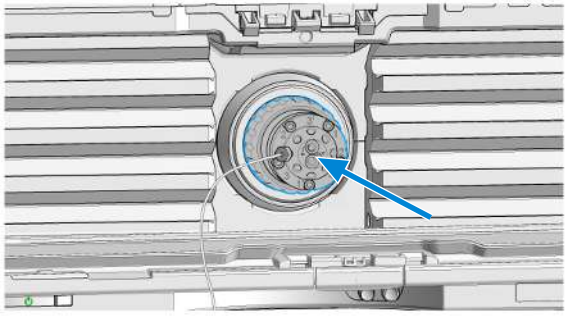
6 Fasten the valve head onto the valve drive using the union nut (see also G7116B_Installation of the Valve Heads).



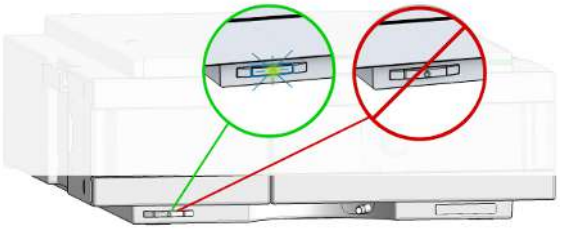
7 Install all required capillary connections to the valve.



8 Push the valve head until it snaps in and stays in the rear position.



9 Switch on the module.



Replace Valve Heads (G1170A)

The following procedure shows installation only. To remove the valve, follow the instructions in reverse order.

NOTE The following procedure exemplarily shows a valve head installation. For correct capillary connections see **Valve topology** in the GUI.

CAUTION **The valve actuator contains sensitive optical parts, which need to be protected from dust and other pollution. Pollution of these parts can impair the accurate selection of valve ports and therefore bias measurement results.**

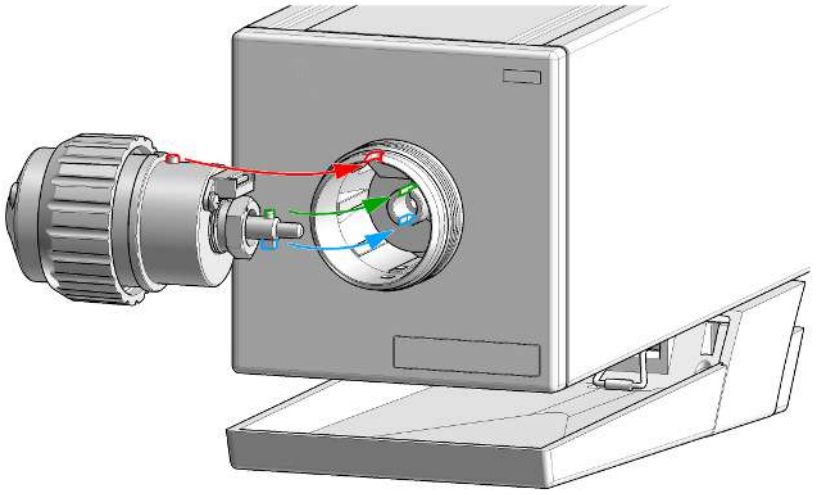
- **Always install a valve head for operation and storage. For protecting the actuator, a dummy valve head can be used instead of a functional valve. Do not touch parts inside the actuator.**

NOTE For a correct installation of the valve head, the outside pin (red) must completely fit into the outside groove on the valve drive's shaft (red). A correct installation is only possible if the two pins (green and blue) on the valve head fit into their corresponding grooves on the valve drive's actuator axis. Their match depends on the diameter of the pin and groove.

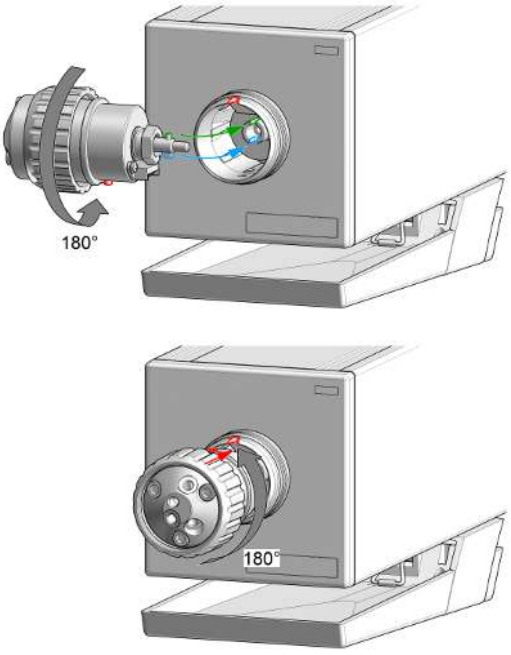
NOTE The tag reader reads the valve head properties from the valve head RFID tag during initialization of the module. Valve properties will not be updated, if the valve head is replaced while the module is on. Selection of valve port positions can fail, if the instrument does not know the properties of the installed valve.

NOTE To allow correct valve identification, power off the module for at least 10 s.

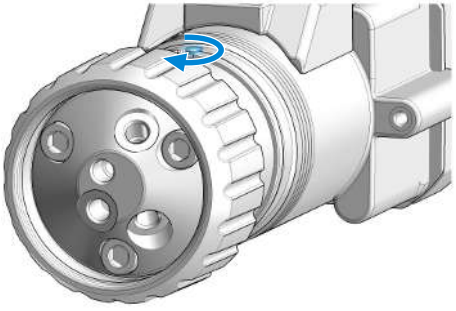
1 Insert the valve head into the valve shaft.



OR: If the outside pin does not fit into the outside groove, you have to turn the valve head until you feel that the two pins snap into the grooves. Now you should feel additional resistance from the valve drive while continuously turning the valve head until the pin fits into the groove.

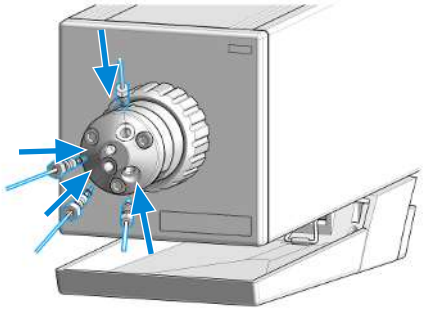


2 When the outer pin is locked into the groove, manually screw the nut onto the valve head.



NOTE Fasten the nut with the 5043-1767 Valve Removal tool.

3 Install all required capillary connections to the valve.



4 Power on or power-cycle your module, so the valve head gets recognized during module initialization.

Replace Parts of Quick Change Valve Head

For bio-inert modules use bio-inert parts only!

Do not mix with bio / biocompatible parts.

For biocompatible modules use bio / biocompatible parts only!

Do not mix with bio-inert parts.

When

- If valve leaks.

Tools required

Qty.	p/n	Description
1		Hexagonal key, 9/64
1		Hexagonal key, 3/32
1		Wrench, 1/4 inch
1		Hexagonal driver SW-6.35 slitted
1		Hexagonal driver SW-4 slitted

1. Remove capillaries from ports.
2. Loosen each fixing stator screw two turns at a time. Remove the bolts from the head.
3. Remove the stator head (and stator face if applicable).
4. Remove the stator ring.
5. Remove the rotor seal (and isolation seal if damaged or contaminated).
6. Install the new isolation seal (if required). Ensure the metal spring inside the ring faces towards the valve body.
7. Install the new rotor seal.
8. Replace the stator ring. Ensure the stator ring is flush with the valve body.

9 Place the new (if required) stator face in place on the stator head. Reinstall the stator head.

10 Insert the stator screws in the stator head. Tighten the screws alternately two turns at a time until the stator head is secure.

11 Reconnect the pump capillaries to the valve ports.

CAUTION

Wrong use of the System Pressure Test may damage valve.

— Always select an appropriate pressure limit for the test. Do not exceed the maximum pressure of pressure sensitive components, for example, set the Maximum Pressure to 800 bar, if an 800 bar Quick Change Valve Head is installed.

12 Perform the System Pressure Test to ensure the valve is leak tight.

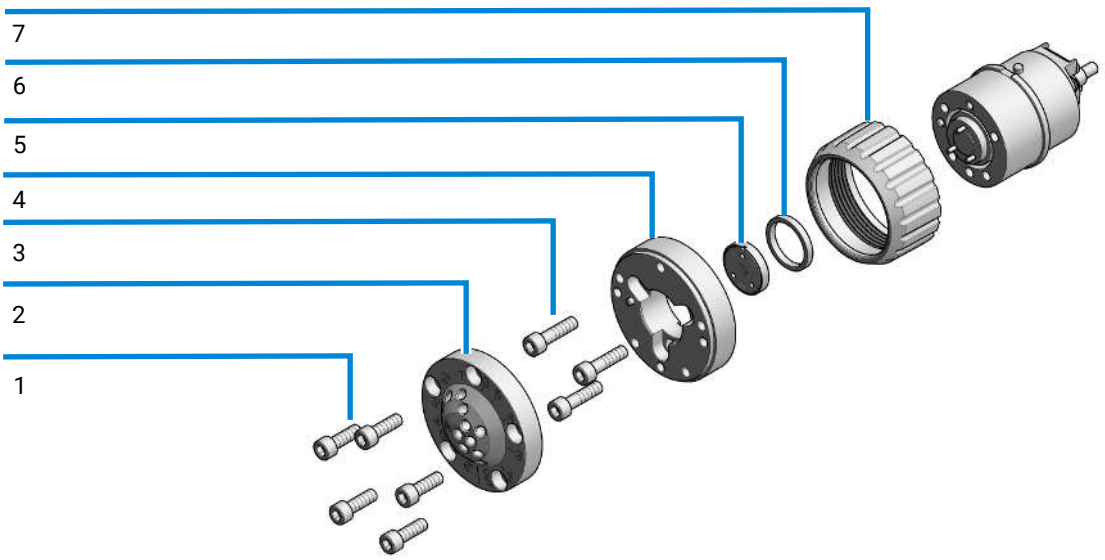


Figure 227: Valve Head Parts (example)

1	Stator screws
2	Stator head assembly
3	Stator ring screws (not available)
4	Stator ring (available for service only)
5	Rotor seal
6	Bearing ring
7	Spanner nut (available for service only)

NOTE

Figure 227 on page 375 illustrates replacement parts for the valve heads, with the 6-column selector valve as an example. The valves can vary in their appearance and do not necessarily include all of the illustrated parts. Neither, every spare part is available for each flavor of the valve.

Replacing the Fuses of the Infinity Valve Drive

When

- If the flow module shows no reaction.

Tools required

Qty.	p/n	Description
1		Screwdriver

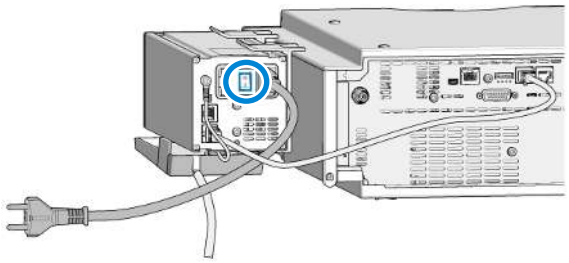
Parts required

Qty.	p/n	Description
2	2110-1486	Fuse 2 AT250 V

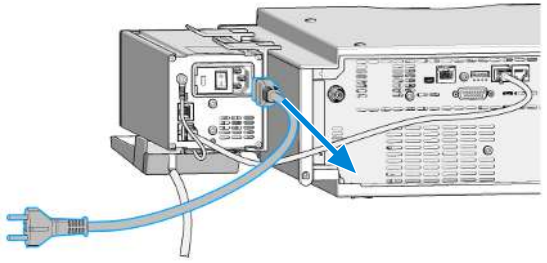
WARNING **Electrical shock**

- **Disconnect the module from line power before changing a fuse or trying to open the hatch of the power input socket.**
- **Never reconnect the line power before havingthe power input socket closed.**

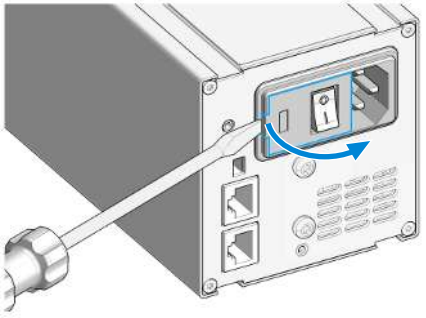
1 Switch off the instrument. The line switch is located at the rear of the module.



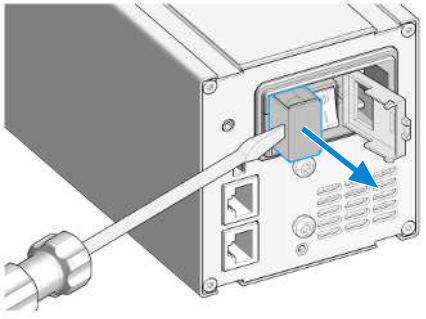
2 Disconnect the power cable from the power input socket.



3 To access the fuse drawer, gently lift the outer plastic housing of the power inlet socket using a flat screwdriver.

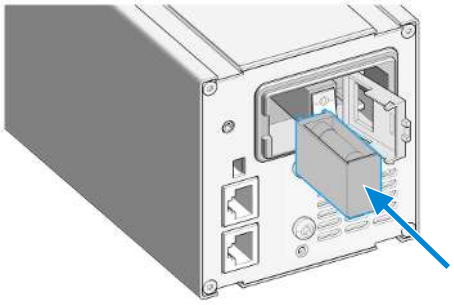


4 Pull out the fuse drawer as shown.

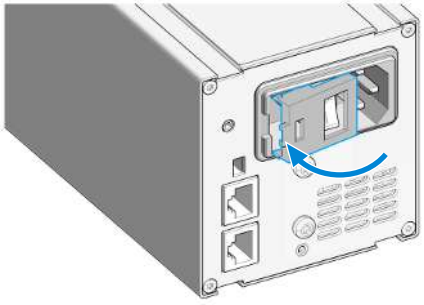


5 Replace the defect fuse(s).

6 Slide in the fuse drawer and push till it fits tightly.



7 Finally, close the fuse drawer housing, reconnect the instrument to the power line and switch it on.



13 Parts for Maintenance

This chapter provides information on parts material required for the solution.

Parts for the 2D-LC System

2D-LC Loops

2D-LC Loops for Standard 2D-LC

p/n	Description
5067-5440	Calibrated loop kit for 2D-LC Internal part number, not orderable
5067-5446	Loop housing kit
5067-5424	20 µL Loop 2D-LC
5067-5425	40 µL Loop 2D-LC
5067-5437	60 µL Loop 2D-LC
5067-5426	80 µL Loop 2D-LC
5004-0036	180 µL Loop 2D-LC
5500-1238	Capillary ST 0.12 mm x 105 mm SL/SL (Bypass Capillary)

2D-LC Loops for MHC valve Fitting M4

p/n	Description
5067-6643	Capillary ST 0.35 x104 mm, M/M, 10
5067-6644	Capillary ST 0.35 x208 mm, M/M, 20 µL
5067-5926	Capillary ST 0.35 x 420 mm M/M 40 µl
5067-6645	Capillary ST 0.35 x831 mm, M/M, 80 µL
5067-6646	Capillary ST 0.35 x1247 mm, M/M, 120 µL
5067-6647	Capillary ST 0.35 x1870 mm, M/M, 180 µL
5067-6141	M4 Blank nut
5023-2504	Hex driver SW-4 slitted

2D-LC Capillaries

1200 Infinity Series 2D-LC Capillary Kit

p/n	Description
5021-1820	Flex capillary, 0.12 mm x 105 mm, no fittings
G1316-87321	Capillary column-heat exchanger 105 mm lg, 0.17 mm i.d.
5021-1822	Capillary, 0.12 mm x 280 mm
5021-1823	Capillary column – detector SST 400 mm lg, 0.12 mm i.d.
5021-1819	Capillary ST 0.17 mm x 400 mm S/S
5065-9964	Capillary ST 0.12 mm x 500 mm
5067-4609	Capillary ST 0.17 mm x 500 mm SX/-
5067-4669	Capillary ST 0.12 mm x 600 mm S/SL
01078-87305	Capillary, 0.17 mm x 80 cm, male fit
5065-4454	Fitting screw long
G1316-60005	Low Dispersion Heat Exchanger Double Assembly
G7116-60015	Quick Connect Heat Exchanger Standard
5500-1188	Quick Turn Capillary ST 0.12 mm x 105 mm, long socket

InfinityLab 2D-LC Capillary Kit legacy

p/n	Description
5067-4651	Capillary ST 0.12 mm x 280 mm SL/SX
5067-4669	Capillary ST 0.12 mm x 600 mm S/SL
5500-1245	Capillary ST 0.17 mm x 400 mm SI/SI
5500-1251	Capillary ST 0.12 mm x 400 mm SL/SL
5500-1240	Capillary ST 0.17 mm x 105 mm SL/SL
5500-1227	Capillary ST 0.17 mm x 150 mm SL-SL
5500-1217	Capillary, ST, 0.17 mm x 900 mm SI/SX
5067-4608	Capillary ST 0.17 mm x 280 mm SX/S
5067-4670	Capillary ST 0.17 mm ID 600 mm pre-swaged

ASM Capillaries

ASM Valve Capillary Replacement Kit

p/n	Description
5500-1300	Capillary ST 0.12 mm x 85 mm M/M (ASM factor 5)
5500-1301	Capillary ST 0.12 mm x 170 mm M/M (ASM factor 3)
5500-1302	Capillary ST 0.12 mm x 340 mm M/M (ASM factor 2)
5500-1303	Capillary ST 0.12 mm x 680 mm M/M (ASM factor 1.5)
5500-1376	Capillary ST 0.12 mm x 170 mm M/M (transfer capillary)

Pressure Release Kit

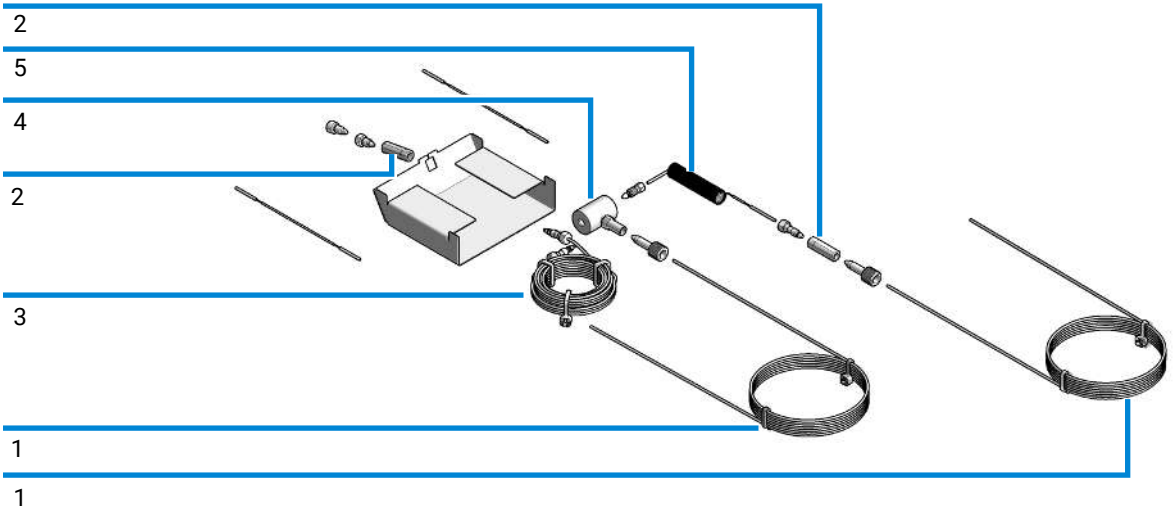


Figure 228: Pressure release kit, parts

#	p/n	Description
	G4236-60010	2D-LC Pressure Release Kit
	0100-0969	Tee, Zero 1/16”SS Low dead volume Not shown
1	5021-1816	Capillary
2	5022-2184	Union, stand LC flow, no fitting
3	G7167-87307	500 µL Loop extension
4	G4212-60022	Pressure Relief Valve
5	5067-5939	Splitter-Capillary 0.05 -ID L-1000 mm

2D-LC Easy Starter Kit

G4236-68100 (2D-LC Easy Starter Kit for ESZ Service) not orderable internal part number

p/n	Description
5190-6895	2D-LC starter sample, 1 x 2 mL
G2453-85060	Formic Acid-Reagent Grade 5 mL (5 cc)
685775-902	InfinityLab Poroshell 120 SB-C18, 2.1 x 100 mm, 2.7 µm
699968-301	Poroshell 120 Bonus-RP, 3.0 x 50 mm, 2.7 µm

G4236-68000 (2D-LC Easy Starter Kit (legacy)) not orderable internal part number

p/n	Description
5190-6895	2D-LC starter sample, 1 x 2 mL
G2453-85060	Formic Acid-Reagent Grade 5 mL (5 cc)
858700-902	RRHD SB-C18, 2.1x100 mm, 1.8 µm, 1200 bar
857768-901	RRHD Bonus-RP, 2.1x50 mm, 1.8 µm, 1200 bar
959757-302	RRHD Eclipse Plus C18, 3.0x50 mm, 1.8 µm

1290 Infinity Valve Drive Parts

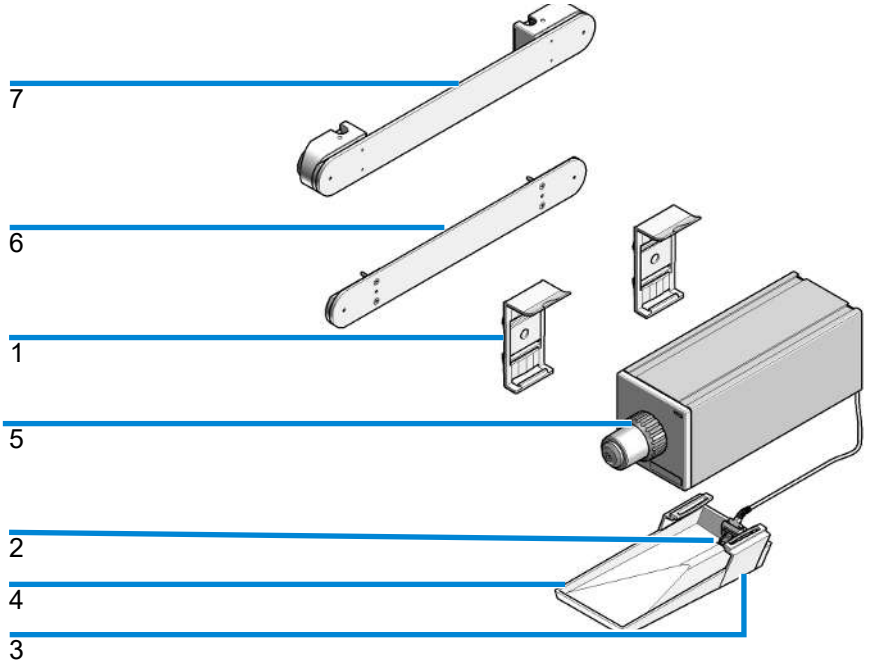


Figure 229: Parts for 1290 Infinity Valve Drive

#	p/n	Description
1	5043-0275	Clamp guide For attaching the valve to a rail assembly
2	5067-4792	Leak sensor assembly External leak sensor
3	5043-0271	Holder leak plane
4	5043-0270	Leak plane
5	5068-0106	Spanner nut
	2110-1486	Fuse 2 AT250 V
6	5067-4634	Valve Rail Kit
7	5067-1510	Rail assy for column organizer

1290 Infinity III Valve Drive Parts

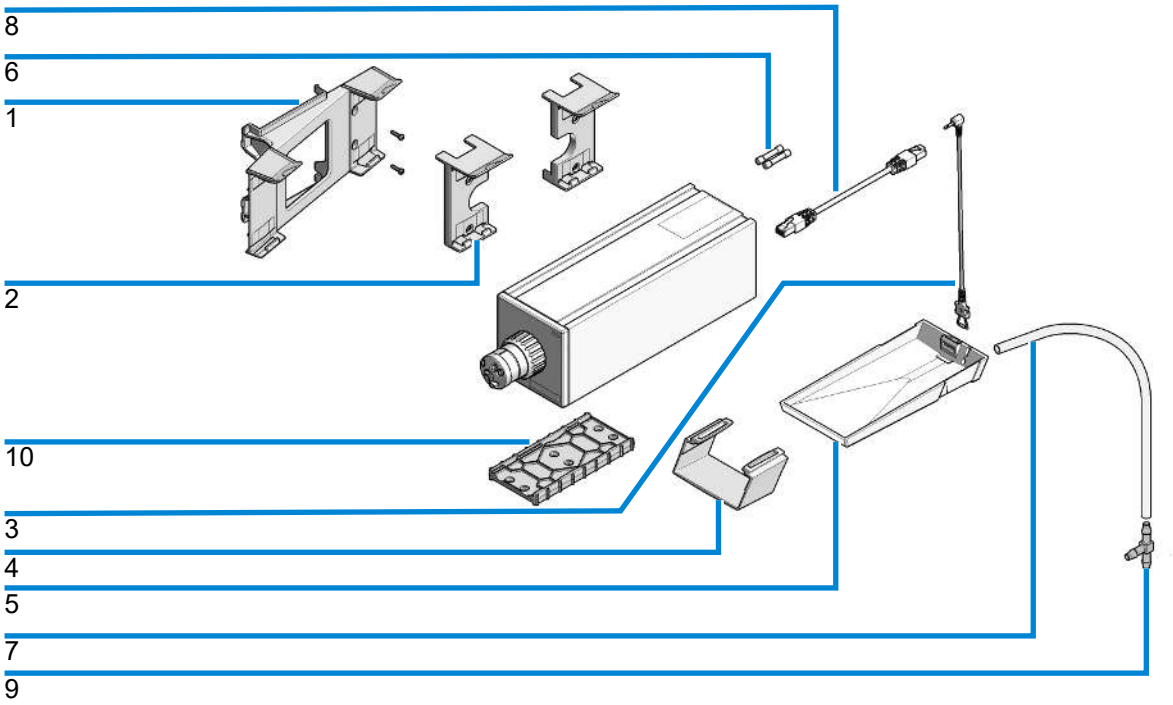


Figure 230: Parts for 1290 Infinity II/III Valve Drive

#	p/n	Description
1	5067-6138	Infinity II & III Valve Holder Kit Right For G7116A/B
	5067-6139	Infinity II & III Valve Holder Kit Left For G7116A/B (Not shown)
2	5067-5685	Clamp Guide Kit
3	5067-4792	Leak sensor assembly External leak plane
4	5043-0271	Holder leak plane
5	5043-0270	Leak plane
6	2110-1486	Fuse 2 AT250 V
7	5063-6527	Tubing, Silicon Rubber, 1.2 m, ID/OD 6 mm/9 mm

#	p/n	Description
8	5181-1519	CAN cable, Agilent module to module, 1 m
9	5500-1156	T-Tube Connector ID6.4
10	5043-0269	Adapter-profile For G1170A (Multiple valve drives can be connected with adapter profiles)

Valve Head Parts

Valve Head Parts

NOTE

The figure below illustrates replacement parts for the valve heads, with the 12-position/13-port Selector valve as an example. The valves can vary in their appearance and do not necessarily include all of the illustrated parts. Neither, every spare part is available for each flavor of the valve.
Use the tables (Valve Options Overview (G7116B) and Valve Options Overview (G7116A)) for identification of the required part numbers.

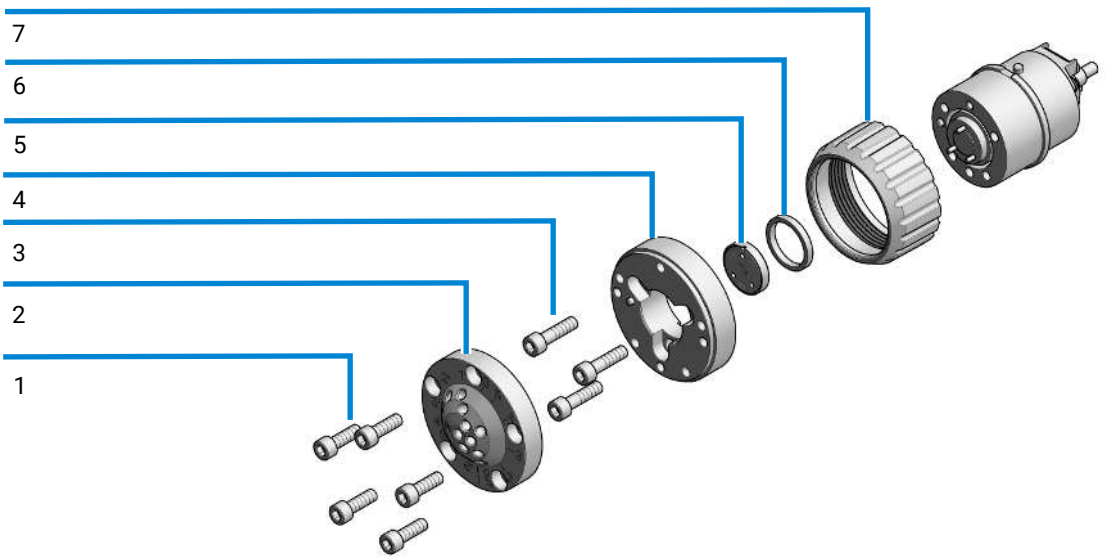


Figure 231: Valve Head Parts (example)

1	Stator screws
2	Stator head assembly
3	Stator ring screws (not available)
4	Stator ring (available for service only)
5	Rotor seal
6	Bearing ring
7	Spanner nut (available for service only)

Technical Specifications

Table 42: Technical specifications

Max. Pressure:	1300 bar
Liquid Contacts:	Stainless Steel, PEEK
Connections:	Accepts 10-32 male threaded and M4 fittings

Tools

Tool for extra fittings

p/n	Description
8710-2462	Hex Key Driver 3/32 inch
5023-2504	Hex driver SW-4 slitted For M4 fittings
5067-6141	M4 Blank nut For plugging unused valve ports
5067-6127	Blank nut SL

Valve Options Overview (for 2D-LC)

The 1300 bar InfinityLab Quick Change Valves are backward compatible to the 1200 bar Valves.

NOTE

The service life of a stator depends on the stress to which the 2D-LC valve is subjected. Therefore, a visual inspection of the surface during maintenance is very important. If scratches or heavy wear is visible during the inspection, the stator must be replaced.

G4136A

2D-LC Valve Kit, Standard

Qty.	p/n	Description
1	5067-4244	2D-LC Valve Head, 1300 bar
1	5067-5440	Calibrated loop kit for 2D-LC
1	5067-6171	Capillary Kit 2D-LC, Infinity Classic (optional) Internal part, not orderable
1	G4236-68100	2D-LC Easy Starter Kit for ESZ Service Internal part, not orderable
2		Multiple Heart-Cutting Valve

Qty.	p/n	Description
1	5067-6585	Capillary Kit 2D-LC, Infinity II/III Internal part, not orderable
1	G1680-63721	Network LAN Switch

5067-4244

2D-LC Valve Head, 1300 bar

Qty.	p/n	Description
3	1535-4857	Stator screws, 10/pk
1	1534-4045	Bearing ring
1	5068-0214	Rotor Seal (VHP)
1	5068-0120	Stator ring
1	5068-0115	Stator

G4243A

2D-LC Valve Kit, ASM

Qty.	p/n	Description
1	5067-4266	2D-LC ASM Valve Head, 1300 bar
1	G4236-68100	2D-LC Easy Starter Kit for ESZ Service Internal part, not orderable
1	G1680-63721	Network LAN Switch
1	5500-1300	Capillary ST 0.12 mm x 85 mm M/M
1	5500-1301	Capillary ST 0.12 mm x 170 mm M/M
1	5500-1302	Capillary ST 0.12 mm x 340 mm M/M
1	5500-1303	Capillary ST 0.12 mm x 680 mm M/M
4	5500-1376	Capillary ST 0.12 mm x 170 mm M/M (Transfer Capillary)

5067-4266

2D-LC ASM Valve Head, 1300 bar

p/n	Description
5068-0019	Stator screws
5068-0257	Bearing Ring
5068-0240	Rotor Seal (VHP)
5068-0239	Stator
	(not included)

Multiple Heart-Cutting Valve

Qty.	p/n	Description
1	5067-4273	6-column selector valve head, 1300 bar
6	5067-5926	Capillary ST 0.35 x 420 mm M/M 40 µl
2	5500-1270	Capillary ST 0.12 mm x 170 mm S/M (Transfer Capillary)
1	5043-0269	Adapter-profile

5067-4273

6-column selector valve head, 1300 bar

Qty.	p/n	Description
5	5068-0089	Stator screws
1	1534-4045	Bearing ring
1	5068-0242	Rotor Seal (PEEK)
1	5068-0241	Stator Head

Obsolete Valve Heads

The following 1200 bar valve heads are no longer orderable:

5067-4214

2D-LC Valve 1200 bar legacy

p/n	Description
5068-0186	Rotor Seal (Vespel)
5068-0115	Stator
1535-4857	Stator screws, 10/pk
1534-4045	Bearing ring

Multiple Heart-Cutting Valve legacy

Qty.	p/n	Description
1	5067-4142	6 Column selector valve head, 1200 bar
6	5067-5926	Capillary ST 0.35 x 420 mm M/M 40 µl
1	5974-0197	Capillary Cover Label
2	5067-5113	Capillary ST 0.17 mm x 250 mm SL/M
2	5067-6188	Capillary ST 0.17 mm x 500 mm SL-M

5067-4142

6-Column selector valve 1200 bar legacy

p/n	Description
5068-0077	Stator Head
5068-0067	Rotor seal (6 Column Selector 1200 bar)
5068-0089	Stator screws
1534-4045	Bearing ring

MS Diverter Valve

G4231A

2-position/6-port valve head, 800 bar

p/n	Description
5067-4282	2-position/6-port valve head, 800 bar
5067-4730	2-position/6-port Cap Kit 0.17 mm
5067-4249	2-position/6-port Cap Kit 0.12 mm, incl. QC-HEx
5067-4250	2-position/6-port Cap Kit 0.12 mm, incl. LD-HEx
5067-6597	2-position/6-port Cap Kit 0.17 mm, incl. QC-HEx

Alternative diverter valves (2 position / 6 port, PEEK Rotor Seal)

p/n	Description
5067-4137	2ps/6pt valve head, 600 bar
5067-4282	2-position/6-port valve head, 800 bar
0101-1409	Rotor seal (PEEK, 2pos/6port CSV, 600 bar)

Alternative diverter valves (2-position/10-port, PEEK Rotor Seal)

p/n	Description
5067-4145	2-position/10-port valve head, 600 bar
5067-4283	2-position/10-port valve, 800 bar
0101-1415	Rotor Seal (2pos/10port 600 bar)

Alternative diverter valves (2-position/6-port, Vespel Rotor Seal)

p/n	Description
5067-4117	2-position/6-port ultra high pressure valve head, 1200 bar
5068-0008	Rotor seal (2pos/6 port 1200 bar)

Alternative diverter valves (2-position/10-port, Vespel Rotor Seal)

p/n	Description
5067-4118	2-position/10-port ultra high pressure valve head, 1200 bar
5068-0012	Rotor seal (2pos/10port 1200 bar)

Additional Parts for the MS Diverter Valve Setup

p/n	Description
G4212-60022	Pressure Relief Valve
5067-4606	Capillary ST 0.12 mm x 400 mm SX/-
0890-1915	Capillary PK 0.13 mm x 150 cm
5500-1228	Capillary ST 0.3 mm x 80 mm SL-SL
5063-6591	PEEK Fittings 10/PK
0100-0969	Tee, Zero 1/16"SS Low dead volume
5067-6127	Blank nut SL
5062-2462	Tube PTFE 0.7 mm x 5 m, 1.6 mm od

Valve Options Overview (G7116B)

Table 43: Replacement parts standard valve heads for G7116B

Valve Head	Rotor Seal	Stator Head	Stator Screws	Stator Ring
5067-4233 8-Position/18-Port Valve 1300 bar	5068-0200 (PEEK)	5068-0199	5068-0089	n.a.
5067-4241 2-Position/6-Port Valve 1300 bar	5068-0207 (PEEK)	5068-0006	1535-4857	5068-0120
5067-4240 2-Position/10-Port Valve 1300 bar	5068-0205 (PEEK)	5068-0011	5068-0019	n.a.
5067-4273 6-Position/14-Port Valve 1300 bar	5068-0242 (PEEK)	5068-0241	5068-0089	n.a.
5067-4284 6-Position/14-Port Valve 800 bar	5068-0298 (PEEK)	5068-0241	5068-0089	n.a.

Valve Head	Rotor Seal	Stator Head	Stator Screws	Stator Ring
5067-6682 2-Position/10-Port Valve Bio 1300 bar	5068-0205 (PEEK)	5068-0286	5068-0019	n.a.
5067-4237 8-Position/9-Port Valve 1300 bar	5068-0202 (PEEK)	5068-0001	1535-4857	5068-0120

Obsolete Valve Heads

The following 1200 bar valve heads are no longer orderable:

Table 44: Replacement parts obsolete valve heads for G7116B

Valve Head	Rotor Seal	Stator Head	Stator Screws	Stator Ring
5067-4121 8-Position/9-Port Valve 1200 bar	5068-0002 (Vespel)	5068-0001	1535-4857	5068-0127
5067-4117 2-Position/6-Port Valve 1200 bar	5068-0008 (Vespel)	5068-0006	1535-4857	5068-0127
5067-4118 2-Position/10-Port Valve 1200 bar	5068-0012 (Vespel)	5068-0011	5068-0019	n.a.
5067-4142 6-Position/14-Port Valve 1200 bar	5068-0067 (Vespel)	5068-0077	5068-0089	n.a.

Additional Heater Devices

Table 45: Heat Exchanger Overview

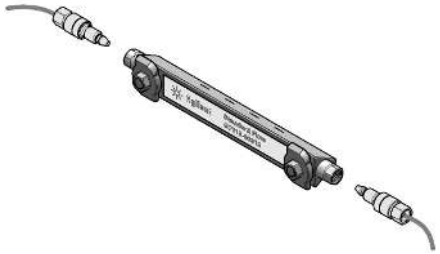
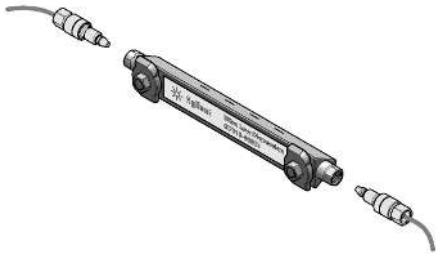
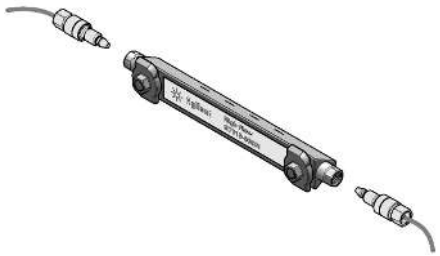
Flow rate	0.075 mm i.d. capillary	0.12 mm i.d. capillary
< 2 mL/min	*Ultra-low Dispersion* G7116-60021 (Internal volume: 1.0 µL)	*Standard Flow* G7116-60015 (Internal volume: 1.6 µL)
> 2 mL/min		*High Flow* G7116-60031 (Internal volume: 3.0 µL)

For details, see Table 46 on page 396.

Additional Heater Devices (for G7116B)

Blank heater assemblies without capillaries and fittings:

Table 46: InfinityLab Quick Connect Heat Exchanger

Item	Description
	G7116-60015 (Quick Connect Heat Exchanger Standard)
	G7116-60021 (Quick Connect Heat Exchanger Ultra Low Dispersion) **NOTE:** Use InfinityLab Quick Turn Fittings to connect to the Quick Connect Heat Exchanger Ultra Low Dispersion.
	G7116-60031 (Quick Connect Heat Exchanger High Flow)

Accessories and Consumables (for G7116B)

G7116-68705 Accessory Kit (for G7116B)

The Accessory Kit (for G7116B) contains accessories and tools needed for the installation and maintenance.

p/n	Description
5181-1516	CAN cable, Agilent module to module, 0.5 m

p/n	Description
5063-6527	Tubing, Silicon Rubber, 1.2 m, ID/OD 6 mm/9 mm
5500-1191	InfinityLab Quick Turn Capillary ST 0.12 mm x 280 mm, long socket
5067-5966	InfinityLab Quick Turn Fitting
5067-5957	InfinityLab Quick Connect Assy ST 0.12 mm x 105 mm
G7116-60015	Quick Connect Heat Exchanger Standard
G7116-68003	Column Holder Lamella, 2/pk (delivered as a part of G7116-60015)
5043-0915	Fitting mounting tool
G7116-60006	Divider Assembly MCT
5022-2184	Union, stand LC flow, no fitting
	Double Drain Connector

Available Consumables (for G7116B)

p/n	Description
G7116-68003	Column Holder Lamella, 2/pk
G7116-68004	Column Holder Clamp, 2/pk
5500-1191	InfinityLab Quick Turn Capillary ST 0.12 mm x 280 mm, long socket Capillary from column outlet to DAD, no fittings.
G7116-60006	Divider Assembly MCT For separating different temperature zones between left and right heater elements.
5067-5917	InfinityLab Column Identification Tag Blank column ID tag (column ID tag reader kit is required)
G7116-60013	InfinityLab Thermal Equilibration Device

Number Kit

p/n	Description
5067-6654	Number Kit 1-8 colored Column Info in red, blue, green, cyan, yellow, black, white, and gray

InfinityLab Quick Connect and Quick Turn Fittings

For further info check either the consumables catalog or Important Customer Web Links on page 151.

InfinityLab Quick Connect Fittings

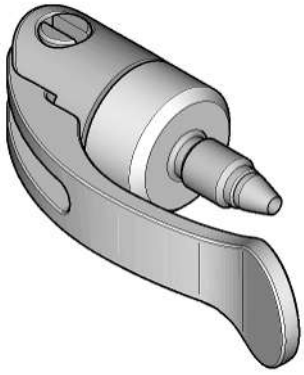


Figure 232: InfinityLab Quick Connect Fitting

p/n	Description
5067-5965	InfinityLab Quick Connect LC fitting (fitting without preinstalled capillary)
5043-0924	Front Ferrule for Quick Connect/Turn Fitting
5067-5961	InfinityLab Quick Connect Assy ST 0.075 mm x 105 mm
5067-6163	InfinityLab Quick Connect Assy ST 0.075 mm x 150 mm

p/n	Description
5067-6164	InfinityLab Quick Connect Assy ST 0.075 mm x 220 mm
5067-6165	InfinityLab Quick Connect Assy ST 0.075 mm x 280 mm
5067-5957	InfinityLab Quick Connect Assy ST 0.12 mm x 105 mm
5067-5958	InfinityLab Quick Connect Assy ST 0.12 mm x 150 mm
5067-5959	InfinityLab Quick Connect Assy ST 0.12 mm x 220 mm
5067-5960	InfinityLab Quick Connect Assy ST 0.12 mm x 280 mm
5067-6166	InfinityLab Quick Connect Assy ST 0.17 mm x 105 mm
5067-6167	InfinityLab Quick Connect Assy ST 0.17 mm x 150 mm
5067-6168	InfinityLab Quick Connect Assy ST 0.17 mm x 220 mm
5067-6169	InfinityLab Quick Connect Assy ST 0.17 mm x 280 mm

InfinityLab Quick Connect Fitting Replacement Capillaries

p/n	Description
5500-1174	InfinityLab Capillary ST 0.075 mm x 105 mm
5500-1175	InfinityLab Capillary ST 0.075 mm x 150 mm
5500-1176	InfinityLab Capillary ST 0.075 mm x 220 mm
5500-1177	InfinityLab Capillary ST 0.075 mm x 250 mm
5500-1178	InfinityLab Capillary ST 0.075 mm x 280 mm
5500-1173	InfinityLab Capillary ST 0.12 mm x 105 mm
5500-1172	InfinityLab Capillary ST 0.12 mm x 150 mm
5500-1171	InfinityLab Capillary ST 0.12 mm x 220 mm
5500-1170	InfinityLab Capillary ST 0.12 mm x 280 mm
5500-1179	InfinityLab Capillary ST 0.12 mm x 400 mm
5500-1180	InfinityLab Capillary ST 0.12 mm x 500 mm
5500-1181	InfinityLab Capillary ST 0.17 mm x 105 mm
5500-1182	InfinityLab Capillary ST 0.17 mm x 150 mm
5500-1183	InfinityLab Capillary ST 0.17 mm x 220 mm
5500-1230	InfinityLab Capillary ST 0.17 mm x 280 mm
5500-1231	InfinityLab Capillary ST 0.17 mm x 500 mm

p/n	Description
5500-1259	InfinityLab Capillary ST 0.25 mm x 150 mm
5500-1260	InfinityLab Capillary ST 0.25 mm x 400 mm

InfinityLab Quick Turn Fitting

Figure 233: InfinityLab Quick Turn Fitting

p/n	Description
5067-5966	InfinityLab Quick Turn Fitting
5043-0924	Front Ferrule for Quick Connect/Turn Fitting

Capillary Kits

NOTE

Further capillary kits can be found in the *Agilent InfinityLab LC Series 1290 Infinity III Valve Drive and Valve Heads User Manual (G1170-Valve-Drive-Heads-en-UseMa-SD-29000412.pdf, SD-29000412)* or on the webpage.

Table 47: Common capillary kit

Part Number	Connection	Amount
5067-4647 (SST-Capillary 340 x 0.12 mm ps ps 1sh 1xlg)	Autosampler to valve	1
5067-4648 (SST-Capillary 700 x 0.17 mm ps ps 1sh 1xlg)	^{2}D pump to valve	1
5067-4649 (Capillary ST 0.12 mm x 90 mm S/SX)	Valve to heat exchanger	2
5067-4650 (Capillary ST 0.12 mm x 150 mm SL/SX)	Short column to valve	2
5067-4651 (Capillary ST 0.12 mm x 280 mm SL/SX)	Long column to valve	2
5067-4652 (SST-Capillary 120 x 0.12 mm ps ps 1xlg 1xlg)	Valve to valve	1
5067-4653 (Capillary ST 0.12 mm x 200 mm S/SX)	Valve to detector	1
0890-1713 (Tubing, PTFE, ID/OD 0.8 /1.6 mm)	Valve to waste	2 m
G1375-87326 (Waste tube, FEP, 1.6 mm od, 0.8 mm id)(includes M4 PEEK fitting)	Valve to waste	1
0100-1259 (Plug-Screw 1032- Fitting)		4
9222-0518 (Bag - plastics)		1

Parts for the Bio 2D-LC System

Install Biocompatible Capillaries

BIO

For biocompatible modules use bio / biocompatible parts only!

Do not mix with bio-inert parts.

Identification of the biocompatible capillaries:

- Biocompatible capillaries are made of MP35N material
- Capillaries look similar to standard stainless steel capillaries
- MP35N capillaries are marked with an orange stripe on the PTFE tube
- The other color of the PTFE tube codes the inner diameter

Figure 234: Color code for biocompatible capillaries

For correct installation of capillary connections it's important to choose the correct fittings, see Syntax for Capillary Description.

CAUTION

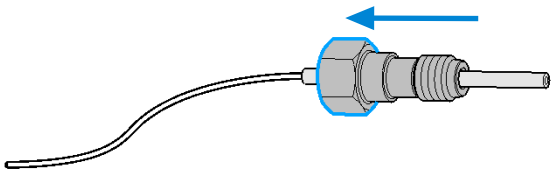
MP35N is harder than stainless steel.

Damage to the gold-plated ferrule.

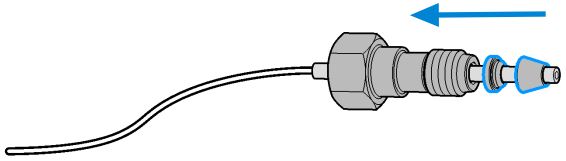
— Do not overtighten the capillaries (finger tight + first resistance with the key + ¼ of a turn with the key).

1 Select a nut that is long enough for the fitting you'll be using.

2 Slide the nut over the end of the tubing or capillary.



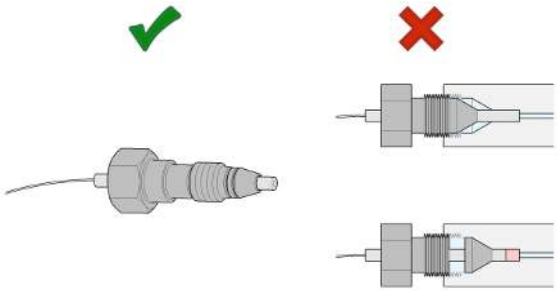
3 Carefully slide the ferrule components on after the nut and then finger-tighten the assembly while ensuring that the tubing is completely seated in the bottom of the end fitting.



4 Use a stable port installed to the module to gently tighten the fitting facing to the module. Or use the column to tighten the fitting facing to the column. This measure forces the ferrule to seat onto the tubing or capillary.

NOTE Do not overtighten. Over-tightening will shorten the lifetime of the fitting.

5 Loosen the nut and verify that the ferrule is correctly positioned on the tubing or capillary.

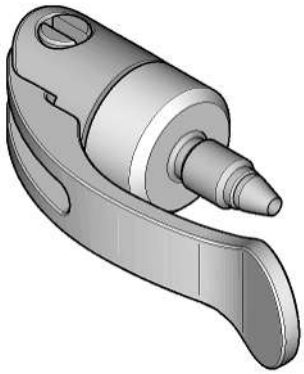


NOTE The first time that the swagelock fitting is used on a column or an injection valve, the position of the ferrule is permanently set. If changing from a column or an injection valve to another, the fitting may leak or decrease the quality of the separation by contributing to band broadening.

Fittings

NOTE

InfinityLab Quick Connect fittings



InfinityLab Quick Connect fittings are truly "finger-tight," reusable fittings for UHPLC, leak-free to 1300 bar. (No tools required) Simply use your fingers to close the lever for a perfect connection every time. This fitting is perfect for the column inlet (closing the lever is equivalent to 1 complete turn of a wrench).

InfinityLab Quick Turn fittings

With InfinityLab Quick Turn fittings, you will get either a finger-tight connection (leak-free to 400 bar), or a UHPLC connection (leak-free to 800 bar with mounting tool p/n 5043-0915, and 1300 bar after a quarter turn of a wrench). The spring-loaded design guarantees zero-dead-volume and makes it ideal for connections at the column outlet and detector.

For details, see *Agilent InfinityLab: Making Great Connections – Less stress, more reliable fittings* (https://www.agilent.com/en/products/liquid-chromatography/lc-supplies/capillaries-fittings/infinitylab-fittings/agilent-infinitylab-fittings-video).

Bio 2D-LC Loops

For biocompatible modules use bio / biocompatible parts only!

Do not mix with bio-inert parts.

Bio Loops for SHC and MHC valve Fitting M4

p/n	Description
5004-0025	Capillary MP35N 0.35 mm x 104 mm M/M 10 µL
5004-0026	Capillary MP35N 0.35 mm x 208 mm M/M 20 µL
5004-0027	Capillary MP35N 0.35 mm x 420 mm M/M 40 µL
5004-0028	Capillary MP35N 0.35 mm x 831 mm M/M 80 µL
5004-0029	Capillary MP35N 0.35 mm x 1247 mm M/M 120 µL
5004-0030	Capillary MP35N 0.35 mm x 1870 mm M/M 180 µL

Bio 2D-LC Capillaries

For biocompatible modules use bio / biocompatible parts only!

Do not mix with bio-inert parts.

InfinityLab Bio 2D-LC Capillary Kit (5005-0077)

Qty.	p/n	Description
3	5500-1603	Quick Turn Capillary MP35N 0.17 mm x 400 mm
1	5004-0031	Capillary MP35N 0.12 mm x 600 mm
2	G7116-60071	Quick Connect Bio Heat Exchanger Standard Flow

Qty.	p/n	Description
2	5500-1578	Quick Connect Capillary MP35N 0.12 mm x 105 mm
2	5500-1597	Quick Turn Capillary MP35N 0.12 mm x 400 mm
1	5500-1599	Quick Turn Capillary MP35N 0.17 mm x 105 mm
1	5500-1600	Quick Turn Capillary MP35N 0.17 mm x 150 mm
1	5500-1596	Quick Turn Capillary MP35N 0.12 mm x 280 mm
2	5067-5965	InfinityLab Quick Connect LC fitting
20	5067-5966	InfinityLab Quick Turn Fitting
1	0890-1713	Tubing, PTFE, ID/OD 0.8 /1.6 mm
1	5063-6591	PEEK Fittings 10/PK

NOTE

InfinityLab Quick Connect fittings are truly "finger-tight," reusable fittings for UHPLC, leak-free to 1300 bar.
No tools required. Simply use your fingers to close the lever for a perfect connection every time. This fitting is perfect for the column inlet (Remember: closing the lever is equivalent to 1 complete turn of a wrench).
With InfinityLab Quick Turn fittings, you will get either a finger-tight connection (leak-free to 400 bar), or a UHPLC connection (leak-free to 800 bar with 5043-0915 (Fitting mounting tool) , and 1300 bar after a quarter turn of a wrench). The spring-loaded design guarantees zero-dead-volume and makes it ideal for connections at the column outlet and detector.

Additional Biocompatible Capillaries

BIO

For biocompatible modules use bio / biocompatible parts only!

Do not mix with bio-inert parts.

Qty.	p/n	Description
1	5500-1596	Quick Turn Capillary MP35N 0.12 mm x 280 mm for short columns
1	5500-1598	Quick Turn Capillary MP35N 0.12 mm x 500 mm for long columns
1	5500-1597	Quick Turn Capillary MP35N 0.12 mm x 400 mm
1	5500-1599	Quick Turn Capillary MP35N 0.17 mm x 105 mm

Qty.	p/n	Description
1	5500-1603	Quick Turn Capillary MP35N 0.17 mm x 400 mm
1	5500-1578	Quick Connect Capillary MP35N 0.12 mm x 105 mm
1	5500-1279	Capillary MP35N 0.12 mm x 500 mm SI/SI
1	5500-1419	Capillary MP35N 0.17 mm x 500 mm, SI/SI
1	5004-0031	Capillary MP35N 0.12 mm x 600 mm
1	5500-1376	Capillary ST 0.12 mm x 170 mm M/M
1	5500-1227	Capillary ST 0.17 mm x 150 mm SL-SL
1	5500-1283	Capillary MP35N 0.25 mm x 80 mm Pressure Sensor to Outlet Filter, to pump head, and to Multipurpose valve
1	5500-1284	Capillary MP35N 0.17 mm x 120 mm SI/SX
1	5004-0041	Capillary MP35N 0.17 mm x 130 mm SI/SX
1	5005-0046	Capillary MP35N 0.12 mm x 2 m
1	5500-1593	Quick Turn Capillary MP35N 0.12 mm x 105 mm
1	5067-5966	InfinityLab Quick Turn Fitting
1	5043-0277	PEEK blank nut for bio-compatible devices

NOTE InfinityLab Quick Turn fittings require the capillaries specified in this table.

NOTE InfinityLab Quick Connect fittings are truly "finger-tight," reusable fittings for UHPLC, leak-free to 1300 bar.
No tools required. Simply use your fingers to close the lever for a perfect connection every time. This fitting is perfect for the column inlet (Remember: closing the lever is equivalent to 1 complete turn of a wrench).
With InfinityLab Quick Turn fittings, you will get either a finger-tight connection (leak-free to 400 bar), or a UHPLC connection (leak-free to 800 bar with , and 1300 bar after a quarter turn of a wrench). The spring-loaded design guarantees zero-dead-volume and makes it ideal for connections at the column outlet and detector.

Pressure Release Kit

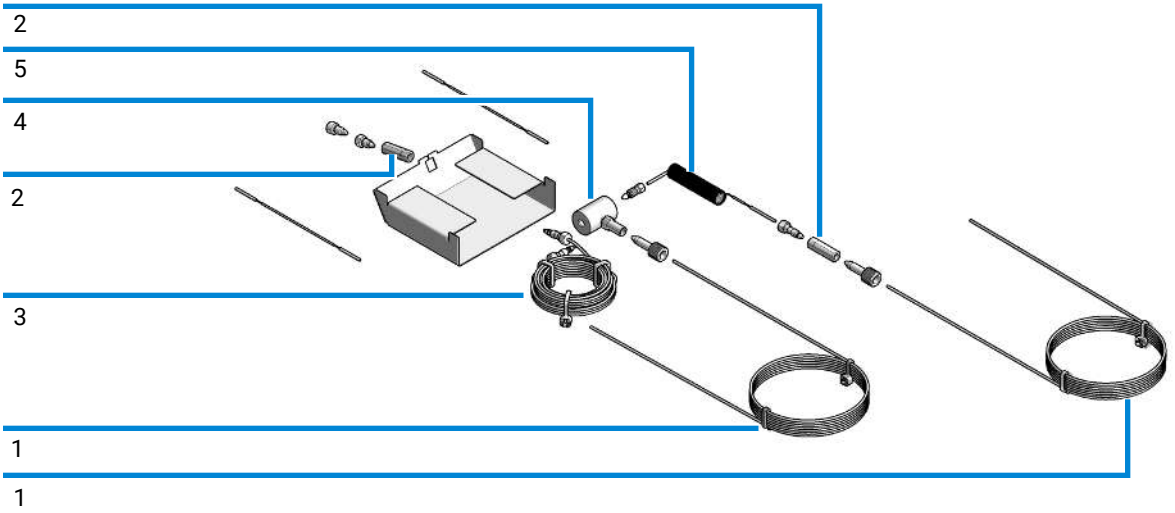


Figure 235: Pressure release kit, parts

#	p/n	Description
	G4236-60010	2D-LC Pressure Release Kit
	0100-0969	Tee, Zero 1/16”SS Low dead volume Not shown
1	5021-1816	Capillary
2	5022-2184	Union, stand LC flow, no fitting
3	G7167-87307	500 µL Loop extension
4	G4212-60022	Pressure Relief Valve
5	5067-5939	Splitter-Capillary 0.05 -ID L-1000 mm
	5022-2144	T-connector, PEEK, 1/16 in, 0.57 µL swept volume

Valve Options Overview (for Bio 2D-LC)

Bio 2D-LC ASM Valve Kit

For biocompatible modules use bio / biocompatible parts only!

Do not mix with bio-inert parts.

InfinityLab Bio 2D-LC ASM Valve Kit (G5643B)

p/n	Description
5005-0078	Agilent InfinityLab Bio 2D-LC ASM Valve
5190-6895	2D-LC starter sample, 1 x 2 mL
G5642-64000	Multiple Heart-Cutting Valve
699968-301	Poroshell 120 Bonus-RP, 3.0 x 50 mm, 2.7 µm
G4236-64000	2D-LC Easy Start USB Media Kit
5005-0077	InfinityLab Bio 2D-LC Capillary Kit
G2453-85060	Formic Acid-Reagent Grade 5 mL (5 cc)
685775-902	InfinityLab Poroshell 120 SB-C18, 2.1 x 100 mm, 2.7 µm
G1680-63721	Network LAN Switch
	Regional power cord

NOTE

The InfinityLab Bio 2D LC ASM Valve Kit (G5643B) that contains the Bio 2D-LC ASM Valve replaces the InfinityLab Bio 2D LC ASM Valve Kit (G5643A) that contained the 2D-LC Valve p/n: 5067-4266.

Bio 2D-LC ASM Valve Kit (1300 bar)

For biocompatible modules use bio / biocompatible parts only!

Do not mix with bio-inert parts.

5005-0078 (Agilent InfinityLab Bio 2D-LC ASM Valve)

p/n	Description
5320-0017	Bio 2D-LC ASM Valve Head, 1300 bar
5004-0021	Capillary MP35N 0.12 mm x 85 mm M4/M4 (ASM factor 5)
5004-0022	Capillary MP35N 0.12 mm x 170 mm M4/M4 (ASM factor 3)
5004-0023	Capillary MP35N 0.12 mm x 340 mm M4/M4 (ASM factor 2)
5004-0024	Capillary MP35N 0.12 mm x 680 mm M4/M4 (ASM factor 1.5)
5004-0020	Capillary MP35N 0.12 mm x 170 mm M4/M4 (transfer capillary)
0890-1713	Tubing, PTFE, ID/OD 0.8 /1.6 mm
5005-0064	Blank Nut, bio-compatible, MP35N, for M4 port (not included)
0100-2441	ZDV Union PEEK with fittings (not included)

ASM-Valve-Head Bio

For biocompatible modules use bio / biocompatible parts only!

Do not mix with bio-inert parts.

Qty.	p/n	Description
1	5068-0257	Bearing Ring
1	5068-0240	Rotor Seal (VHP)
5	5068-0019	Stator screws
1	5299-0005	Stator 5-10 PD CF 1300 bar BIO

Multiple Heart-Cutting Valve

For biocompatible modules use bio / biocompatible parts only!

Do not mix with bio-inert parts.

Qty.	p/n	Description
1	5067-4273	6-column selector valve head, 1300 bar
6	5004-0027	Capillary MP35N 0.35 mm x 420 mm M/M 40 µL Transfer Capillary
1	5043-0269	Adapter-profile

NOTE The current version of this MHC valve uses biocompatible sample loops and a biocompatible valve head.

2-Position/10-Port valve Bio (1300 bar)

For biocompatible modules use bio / biocompatible parts only!

Do not mix with bio-inert parts.

G5641A (2-position/10-port valve, bio 1300 bar) PEEK, MP35N

p/n	Description
5067-6682	2-position/10-port bio valve head, 1300 bar
5068-0286	Stator MP35N
5068-0205	Rotor Seal (PEEK)
5068-0019	Stator screws
5013-0002	Bio 2/10 Capillary Kit 1300 bar (separately orderable)

12-Position/13-Port Selector Valve Head Bio-Inert (210 bar)

For bio-inert modules use bio-inert parts only!

5067-4159 (12-position/13-port selector valve head, 210 bar, bio-inert)

Qty.	p/n	Description
4	5068-0059	Stator screws
1	1535-4045	Bearing ring
1	0101-1288	Rebuild kit (Rotor seal and stator face) (Bio-inert, 12pos/13port selector 210 bar)
1	5068-0097	Bio-inert stator head

2-Position/6-Port Valve Bio-inert (600 bar)

For bio-inert modules use bio-inert parts only!

5067-4148 (2-position/6-port Bio-inert valve, 600 bar)

p/n	Description
5068-0060	Bio-inert stator head
0101-1409	Rotor seal (PEEK, 2pos/6port CSV, 600 bar)
0100-1851	Stator face assy (2pos/6port, 600 bar, Bio-inert)
1535-4045	Bearing ring
5068-0020	Stator Screws, 10/pack

4-Column Selector Valve Bio-inert (600 bar)

For bio-inert modules use bio-inert parts only!

5067-4134 (4-position/10-port Bio-inert valve, 600 bar)

Qty.	p/n	Description
1	5068-0045	Rotor seal (Bio-inert, 4 column selector 600 bar, PEEK)
1	5068-0044	Bio-inert stator head
1	5068-0093	Stator face assy (Bio-inert, 4 column selector 600 bar)
5	5068-0059	Stator screws
1	1534-4045	Bearing ring

Overview of Other Biocompatible Spare Parts of Various Bio-LC Modules

1290 Bio High-Speed Pump (G7132A) Biocompatible Parts

For biocompatible modules use bio / biocompatible parts only!

Do not mix with bio-inert parts.

1290 Bio High-Speed Pump (G7132A) Biocompatible Parts

p/n	Description
G7132-60002	Biocompatible capillary MP35N 0.17 m x 300 mm Purge Valve to Jet Weaver
5500-1421	Biocompatible capillary MP35N 0.25 mm x 130 mm Purge Valve to Pressure Sensor
5500-1420	Biocompatible capillary MP35N 0.25 mm x 250 mm Purge Valve to Pump Head Assemblies channel A and B

p/n	Description
5500-1419	Capillary MP35N 0.17 mm x 500 mm, SI/SI Jet Weaver to Multisampler (Standard Bio-LC Setup)

For further bio pump parts, refer to the user manuals.

1290 Bio Flexible Pump (G7131A/C) Biocompatible Parts

For biocompatible modules use bio / biocompatible parts only!

Do not mix with bio-inert parts.

1290 Bio Flexible Pump (G7131A/C) Biocompatible Parts

p/n	Description
G7131-20009	Metering Seal PTFE (Bio), 100 µL
G7131-60004	Outlet Filter Flex Biocompatible
5500-1283	Capillary MP35N 0.25 mm x 80 mm Pressure Sensor to Outlet Filter, to pump head, and to Multipurpose valve e.g. Pump Head to Pressure Sensor
5500-1419	Capillary MP35N 0.17 mm x 500 mm, SI/SI Purge Valve/Jet Weaver to Multisampler
5500-1284	Capillary MP35N 0.17 mm x 120 mm SI/SX Multipurpose Valve internal connections
5004-0041	Capillary MP35N 0.17 mm x 130 mm SI/SX To/from Jet Weaver
0905-1731	Bio-Inert Wash Seal
5320-0048	Frit for pump outlet filter biocompatible 2/pk
5065-4445	Peristaltic pump cartridge
5720-0020	1290 Infinity II/III Bio Inline Filter Kit

For further bio pump parts, refer to the user manuals.

1290 Bio Multisampler (G7137A) Biocompatible Parts

For biocompatible modules use bio / biocompatible parts only!

Do not mix with bio-inert parts.

1290 Bio Multisampler (G7137A) Biocompatible Parts

p/n	Description
G7137-87201	Needle Bio-compatible
G7137-87012	High pressure seat assembly 0.12 mm Biocompatible
5320-0010	Rotor Seal 1300 bar (PEEK)
G7137-20003	Metering seal 1290 Bio 2 mm piston, 40 µL
5065-4445	Peristaltic pump cartridge
5067-6739	2-position/6-port injection valve Bio 1300 bar
5068-0281	Stator face, MP35N
G7137-60300	Sample Loop MP35N 20 µL, right (red/orange coded)
G7137-60400	Sample Loop MP35N 40 µL, right (green/orange coded)
G7137-60500	Sample Loop MP35N 100 µL, right (blue/orange coded)

Standard

Qty.	p/n	Description
1	5500-1278	Capillary MP35N 0.17 mm x 100 mm SL/SL Analytical Head to Injection Valve
1	5500-1279	Capillary MP35N 0.12 mm x 500 mm SI/SI Injection Valve to Quick Connect Heat Exchanger in MCT
1	5500-1419	Capillary MP35N 0.17 mm x 500 mm, SI/SI Jet Weaver to Multisampler

Multiwash

p/n	Description
5500-1278	Capillary MP35N 0.17 mm x 100 mm SL/SL Analytical Head to Injection Valve
5500-1280	Capillary MP35N 0.17 mm x 250 mm SL-SL Flush Head to Injection Valve

p/n	Description
5500-1279	Capillary MP35N 0.12 mm x 500 mm SI/SI Injection Valve to Quick Connect Heat Exchanger in MCT (Standard Bio-LC Setup)
5500-1419	Capillary MP35N 0.17 mm x 500 mm, SI/SI Jet Weaver to Multisampler (Standard Bio-LC Setup)

For further sampler parts, refer to the user manuals.

1260 Bio Multisampler (G5668A) Bio-Inert Parts

BIO INERT

For bio-inert modules use bio-inert parts only!

1260 Bio Multisampler (G5668A) Bio-Inert Parts

Qty.	p/n	Description
1	G5668-87200	Bio-inert Needle Assembly
1	5068-0209	Rotor Seal (PEEK)
1	G5668-87017	Bio-inert Seat ID 0.17
1	G5668-60500	Bio-inert Sample Loop 100 µL
1	5067-4263	2-position/6-port Injection Valve Bio-inert 600 bar
1	5068-0060	Bio-inert stator head
1	G5611-60500	Capillary 400 x 0.17 mm, titanium (Bio-inert) Pump to Injector (Standard Bio-LC Setup)
1	G5611-60502	Capillary Ti 0.17 mm x 900 mm, L (Bio-inert) Pump to Thermostatted Autosampler (Standard Bio-LC Setup)
1	5043-0277	PEEK blank nut for bio-compatible devices

NOTE

Be careful with installation of stainless steel-cladded PEEK capillaries (Bio-Inert). The capillaries require special attention and different handling compared to usual LC capillaries. See the Technical Note *Installation of stainless steel cladded PEEK capillaries Technical Note (G5611-90120)* for detailed description

Standard

Qty.	p/n	Description
1	5500-1278	Capillary MP35N 0.17 mm x 100 mm SL/SL Analytical Head to Injection Valve
1	5500-1256	Capillary Ti 0.17 mm x 100 mm SL/SL
1	5500-1279	Capillary MP35N 0.12 mm x 500 mm SI/SI Injection Valve to Quick Connect Heat Exchanger in MCT
1	5500-1419	Capillary MP35N 0.17 mm x 500 mm, SI/SI Jet Weaver to Multisampler

Multiwash

Qty.	p/n	Description
1	5500-1278	Capillary MP35N 0.17 mm x 100 mm SL/SL Analytical Head to Injection Valve
1	5500-1280	Capillary MP35N 0.17 mm x 250 mm SL-SL Flush Head to Injection Valve
1	5500-1279	Capillary MP35N 0.12 mm x 500 mm SI/SI Injection Valve to Quick Connect Heat Exchanger in MCT (Standard Bio-LC Setup)
1	5500-1419	Capillary MP35N 0.17 mm x 500 mm, SI/SI Jet Weaver to Multisampler (Standard Bio-LC Setup)
1	5500-1257	Capillary Ti 0.17 mm x 250 mm SL/SL Injection Valve to Flushpump-head
1	5500-1256	Capillary Ti 0.17 mm x 100 mm SL/SL

For further sampler parts, refer to the user manuals.

1260/1290 MCT (G7116A/B) Biocompatible Parts

BIO

For biocompatible modules use bio / biocompatible parts only!

Do not mix with bio-inert parts.

1260/1290 MCT (G7116A/B) Biocompatible Parts

p/n	Description
G7116-60071	Quick Connect Bio Heat Exchanger Standard Flow 1.6 µL
G7116-60081	Quick Connect Bio Heat Exchanger High Flow 3.0 µL
G7116-60091	Quick Connect Bio Heat Exchanger Ultra Low Dispersion 1.0 µL

For further bio MCT parts, refer to the user manuals.

1260/1290 MCT (G7116A) Bio-Inert Parts

For bio-inert modules use bio-inert parts only!

1260/1290 MCT (G7116A) Bio-Inert Parts

p/n	Description
G7116-60009	Quick-Connect Heat Exchanger Bio-inert Standard Flow

For further bio MCT parts, refer to the user manuals.

1260/1290 DAD (G7117A/B) Biocompatible Parts

For biocompatible modules use bio / biocompatible parts only!

Do not mix with bio-inert parts.

1260/1290 DAD (G7117A/B) Biocompatible Parts

p/n	Description
G7117-60020	Max-Light Cartridge Cell LSS (10 mm, V(σ) 1.0 µL) MP35N, PEEK, fused silica
G7117-60101	Aperture

NOTE

Aperture is not compatible with other Max-Light Cartridges.
The Aperture should be installed for analysis of *light-sensitive samples*, which are likely to undergo photodegradation. For further details, check the *Agilent InfinityLab LC Series Diode Array Detectors User Manual (G7117-DAD-UseMa-en-SD-29000132.pdf, SD-29000132)*.

1260/1290 DAD (G7117A/B) Bio-Inert Parts

For bio-inert modules use bio-inert parts only!

1260/1290 DAD (G7117A/B) Bio-Inert Parts

p/n	Description
G5615-60018	Max-Light Cartridge Cell Bio-inert (10 mm, V(s) 1.0 µL)

For further detector parts, refer to the user manuals.

1260 DAD (G7115A) / 1260 MWD (G7165A) Bio-Inert Parts

For bio-inert modules use bio-inert parts only!

1260 DAD (G7115A) / 1260 MWD (G7165A) Bio-Inert Parts

p/n	Description
G5615-60022	Standard flow cell bio-inert, 10 mm, 13 µL, 120 bar (12 MPa) for MWD/DAD, includes 0890-1763 – 0.18 x 1500 mm PEEK capillary and 5063-6591 – PEEK fittings

For further detector parts, refer to the user manuals.

1260/1290 VWD (G7114A/B) Biocompatible Parts

For biocompatible modules use bio / biocompatible parts only!

Do not mix with bio-inert parts.

1260/1290 VWD (G7114A/B) Biocompatible Parts

Qty.	p/n	Description
1	G1314-60189	Bio micro flow cell VWD, 3 mm, Cell Vol. 2 µl, Sapphire, MP35N
1	G1314-60188	Bio standard flow cell VWD, 10 mm, Cell Vol. 14 µl, Sapphire, MP35N

For further detector parts, refer to the user manuals.

1290 FLD (G7121A) Bio-Inert Parts

For bio-inert modules use bio-inert parts only!

1290 FLD (G7121A) Bio-Inert Parts

p/n	Description
G5615-60005	Bio-inert flow cell, 8 µL, 20 bar

For further detector parts, refer to the user manuals.

Selection of Bio LC Columns

For biocompatible modules use bio / biocompatible parts only!

Do not mix with bio-inert parts.

p/n	Description
653750-902	AdvanceBio Peptide Mapping 120Å, 2.1 x 150 mm, 2.7 µm Peptide mapping (reversed-phase chromatography).
PL1912-1502	PLRP-S 1000 Å, 2.1 mm x 50 mm, 5 µm Analytical prep separations of peptides, proteins, and protein complexes (reversed-phase chromatography)
PL1980-3201PK	AdvanceBio SEC 200 Å, 2.1 mm x 150 mm, 1.9 µm, PEEK Aggregation and fragment analysis (size exclusion chromatography)

Additional information:

- 653750-902 (AdvanceBio Peptide Mapping, 2.1 x 150 mm) is a regular stainless steel column that is used for high resolution Peptide Mapping. It was used as an example in the following 2D-LC application *Fully Automated Characterization of Monoclonal Antibody Charge Variants Using 4D-LC/MS*.
- PL1912-1502 (PLRP-S 1000Å, 2.1 x 50 mm) is also a regular stainless steel column but there is also a PEEK lined version available (PL1912-1502PK). It was used as an example in the following 2D-LC application *Characterization of Antibody-Drug Conjugates (ADCs) Using 2D-LC and Native MS*.

For further application details please check the application finder for 2D-LC Applications

https://www.agilent.com/en/promotions/applicationfinder

14 Theoretical Background

This chapter gives the theoratical background of 2D-LC and describes the system components (soft- and hardware) of the 2D-LC Solution.

Theoretical Basis of 2D-LC

In 2D-LC, fractions from a chromatographic system (first dimension) are transferred to a second chromatographic separation system (second dimension). So 2D-LC bases on the application of two independent liquid phase separation systems to a sample. 2D-LC is mainly used to improve resolution and sensitivity or to decrease analysis time.

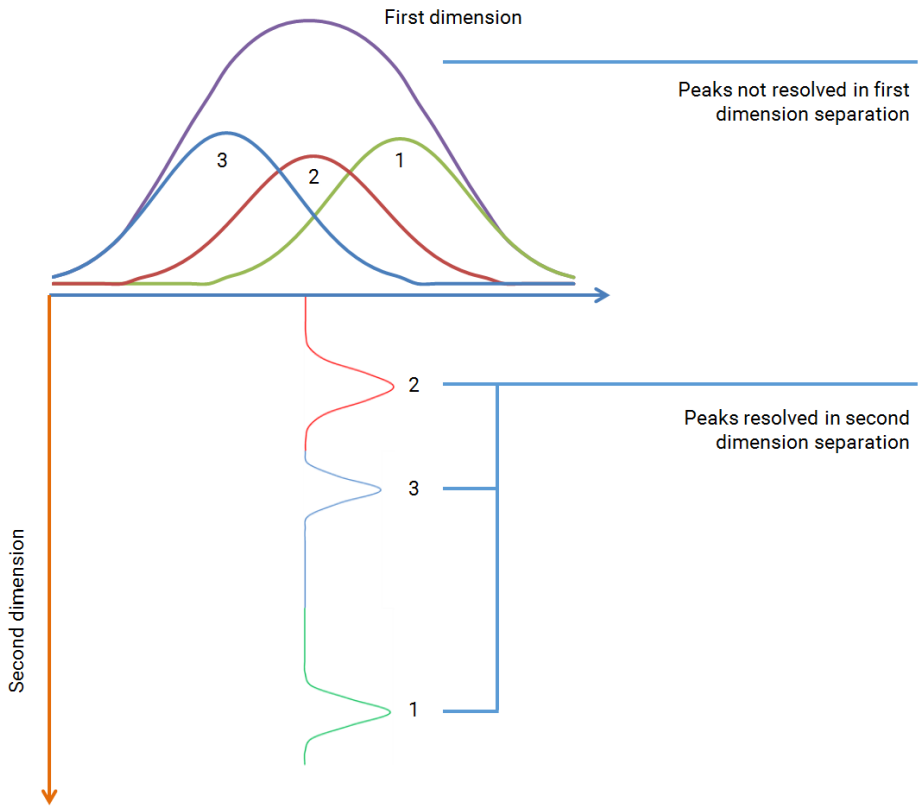


Figure 236: Peak capacity relationship between peak capacities of orthogonal first and second dimension

The most important benefit of 2D-LC over 1D-LC is the increase of resolving power, which is especially important if dealing with complex samples.

For an overview on the main differences between 1D- and 2D-LC, refer to the following topics:

- Orthogonality on page 425,
- Resolution on page 425, and
- Peak Capacity on page 428

The following different methods of 2D-LC exist:

- Heartcutting (LC-LC)

  Only interesting portion of the first dimension effluent transferred to the second dimension.
- Comprehensive (LCxLC)

  Entirety of first dimension effluent sequentially transferred to the second dimension.

Orthogonality

The 2D-LC separation power depends the fact that the two selectivity mechanisms in the different separation stages must be as different as possible. If the mechanisms are completely different and independent the two separations are called *orthogonal*.

Any correlation between the selectivity mechanisms degrades orthogonality and reduces the efficiency of the 2D-LC system.

For strategies to achieve maximum orthogonality, refer to Table 49 on page 434 and Table 50 on page 437.

Resolution

A chromatographic separation can be optimized based on physical parameters of the HPLC column such as particle size, pore size, morphology of the particles, the length and diameter of the column, the solvent velocity, and the temperature. In addition, the thermodynamics of a separation can be considered and the properties of the solute and the stationary and mobile phases (percentage of organic solvent, ion strength, and pH) can be manipulated to achieve the shortest possible retention and highest selectivity.

1D-LC Resolution (R_S) can be described as a function of three parameters:

- Column efficiency or theoretical plates (N),
- Selectivity (α),
- Retention factor (k).

$$R_s = \frac{\sqrt{N}}{4}\left[\frac{\alpha - 1}{\alpha}\right]\left[\frac{k'_2}{k'_2 + 1}\right]$$

Figure 237: Resolution equation

This means that the selection of appropriate mobile and stationary phase properties and temperature is critical in achieving a successful separation.

Resolution in a one-dimensional separation usually is measured with:

$$R = \frac{\Delta t}{4\sigma}$$

R = Resolution
Δt = Difference in retention time maxima of two components
σ = Average standard deviation of two Gaussian peaks

Following results of this formula are important in practice:

- R > 1.5

  Peaks are completely baseline resolved
- R > 1

  Difference in retention time is larger than peak broadening, and therefore peak spacing is adequate to observe distinct component zones
- R < 0.5

  Peaks are completely fused

2D-LC In 2D-LC the separation behaviour is more complex and described below.

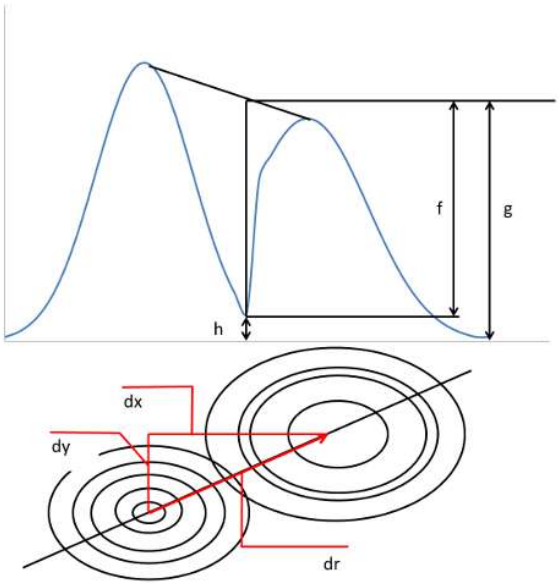


Figure 238: Diagram of ^{2}D resolution measurement: Slice for resolution (top) and 2-dimensional contour plot (bottom)

The distance between two spots in the contour plot may be calculated by the Pythagorean expression:

$$dr = \sqrt{dx^2 + dy^2}$$

For the resolution along the axis of each dimension applies:

$$R_1 = \frac{dy}{4\sigma_1}$$

and

$$R_2 = \frac{dy}{4\sigma_2}$$

So for two dimensions the resolution may be calculated as follows:

$$R_{2D} = \frac{dr}{4\sigma} = \sqrt{\left(\frac{dx}{4\sigma}\right)^2 + \left(\frac{dy}{4\sigma}\right)^2}$$

Figure 239: ^{2}D resolution (pythagorean relation)

or, σ approximated by the average of σ_1 and σ_2, using the easy to measure peak to valley ratio (P = f/g) and assuming that peaks are Gaussian:

$$Rs = \sqrt{-\frac{1}{2} ln\left(\frac{1-P}{2}\right)}$$

Figure 240: ^{2}D resolution (peak to valley ratio relation)

Table 48: Definitions

Symbol	Denotation
R	Resolution
Δt	Difference in retention time maxima of two components
σ	Average standard deviation of Gaussian peaks
dr	Distance between two spots in a plane
P	Peak to valley ratio
f	Difference between amplitude at the valley, h, and g
h	Valley
g	Average peak maximum

Peak Capacity

Peak capacity may be differently defined:

- As the maximum number of peaks that can be resolved in the available separation space (*Geometrical Definition*), or
- As the ratio of the total area of the chromatogram to the area required for the resolution of any zone (*General Definition*)

Geometrical Definition

The peak capacity may be defined as the maximum number of peaks that can be resolved in the available separation space. So peak capacity n_c is related to the number of theoretical plates N:

$$n_c = \mathrm{P}N^{1/2}$$

(P depends on the retention time range)

In practice peaks are usually not distributed randomly over the chromatogram and often overlap. Or in other words: In practice peaks don't fill the available separation space evenly. This is the reason, why the number of detectable components of a sample in 1D-LC is relatively small.

2D-LC separation offers an alternative possibility for increasing n_c: Orthogonal retention mechanisms generate a separation plane. Thus, the peak capacity in 2D-LC is the product of the peak capacities of the individual columns. Due to peak broadening in 1st and 2nd dimension, components in 2D-LC are present as two-dimensional ellipses on the retention plane.

How to calculate n_c depends on the method:

- For comprehensive 2D-LC:

$$n_c = \frac{L_1 L_2}{ab} = n_{c1} n_{c2}$$

L = Separation space for dimension
ab = Area for rectangle circumscribing the ellipse on the separation plane

- For heart-cutting 2D-LC:

$$n_c = \sum_{i=i}^{k} n_{ci}$$

General Definition

Alternatively peak capacity may be defined as the ratio of the total area A of the chromatogram to the area A_0 required for the resolution of any zone:

$$n_{c,alternat} = \frac{A}{A_0}$$

n_c defined that way is related to the geometrical definition by a factor:

$$n_c = \frac{\pi}{4} n_{c,alternat} \approx 0.79 n_{c,alternat}$$

Limits of Peak Capacity in 2D-LC

Under ideal circumstances (*orthogonality*), the overall peak capacity ($n_{c,2D}$) should be equal to the product of the individual peak capacities of the first and second dimension separations (^1n_c and ^2n_c)

$$n_{c,2D} = {}^1n_c \times {}^2n_c$$

In practice the increase in peak capacity is not directly proportional to increase in ability to resolve peaks.

Probable reason for this:

- In 1D-LC, with a baseline width of a single component peak $x_0 = 6\sigma$, x_0 units of component free space on both sides of the maxima is necessary to ensure baseline resolved peaks.
- In 2D-LC the single component zone is $A_0 = 2\pi r^2$ and an area of component free space of $\pi(2r)^2$.
- As a result: For every two component free widths in one dimension, four component free areas are required in two dimensions.

Conclusions for 2D-LC

1D-LC is inadequate for the separation of complex mixtures, as the number of observable peaks compared to number of peaks to observe is too low. One theoretical model (Statistical Model of Overlap = SMO), that correlates well with real world observations, predicts, that the maximal fraction of the total peak capacity that can be seen as chromatographic peaks is 37 % and even only 18 % as single peaks. This implicates that extremely high peak capacities are needed to separate complex samples with lots of components which is extremely difficult to achieve.

Compared to 1D-LC separations, it's complicated to predict the number of observable peaks in 2D-LC. For example, at a given peak capacity and a given number of components, the aspect ratio in the two axes of separation has impact on how effective the two separation are.

From the practical point of view the performance between 1D- and 2D-LC should be compared, considering the following aspects:

- Peak capacity
- Number of peaks observed in experimental chromatograms

Ideal ^{2}D Peak Capacity

One major problem in 2D-LC is loss of ^{1}D resolution due to ^{2}D sampling process. The determining factors are:

- Gradient time of the ^{2}D separation cannot exceed the sampling interval of the ^{1}D separation
- Resolution of a pair of peaks in the two-dimensional space is related to the resolution on the first and second dimensions as the Pythagorean average (see Figure 238 on page 427)

A ^{2}D chromatogram is only a way of displaying a lengthy series of sequential chromatograms obtained on the second column and the second column and detector are just a unique type of chemically selective detector of what comes out of the first column (see ^{2}D as detector on page 432). The peak width observed on the second column is independent of the sampling time used in the ^{1}D.

This leads to two extreme scenarios, on how mixtures of components may behave:

- Unresolved mixture is injected into second column and second column separates analytes perfectly

  $R_{s,2D}$ is independent of ^{1}D sampling rate
- Partially resolved mixture is injected into second column and analytes co-elute on the second column

  $R_{s,2D}$ strongly depends on first dimension sampling rate.

  This indicates, that it's very important to respect, how often the ^{1}D effluent must be sampled to avoid loss of resolution.

NOTE

The theoretical limits for ideal ^{2}D peak capacity are defined by the Murphy-Schure-Foley Criterion (M-S-F sampling criterion). According to this criterion, the effluent must be sampled at least 3 – 4 times over 8σ width of the first dimension peak.

^{2}D as detector

Functionally the ^{2}D of 2D-LC operates like a chemically sensitive detector for the peaks that elute from the ^{1}D column. Thus, 2D-LC may be understood as a three step process:

- ^{1}D separation (1)
- Sampling of the ^{1}D (2)
- ^{2}D separation and detection (3)

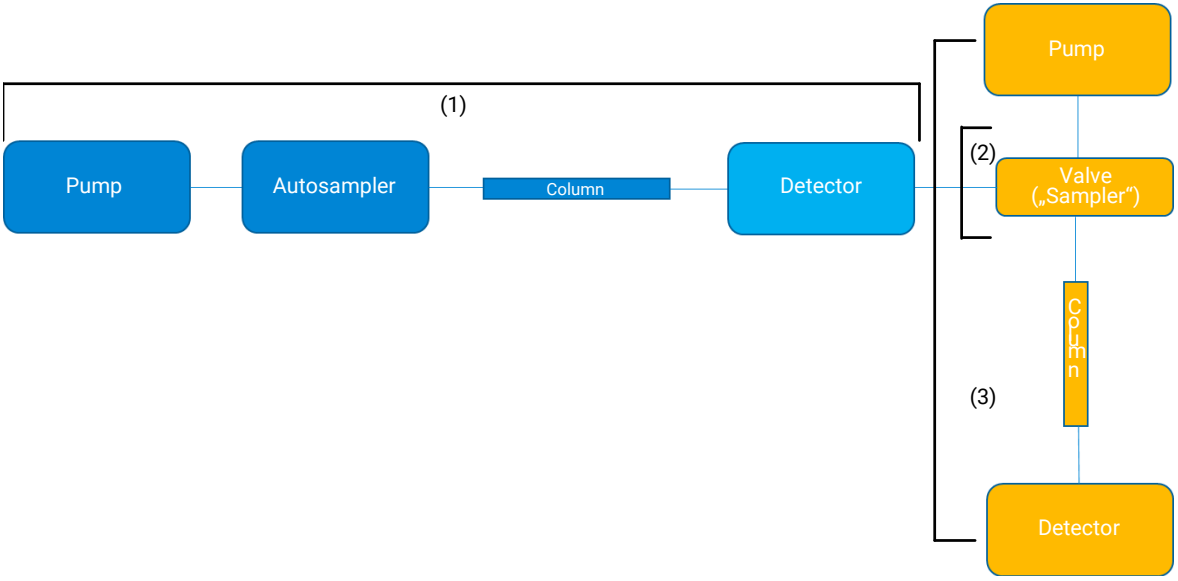


Figure 241: Diagram of instrumentation for 2D-LC

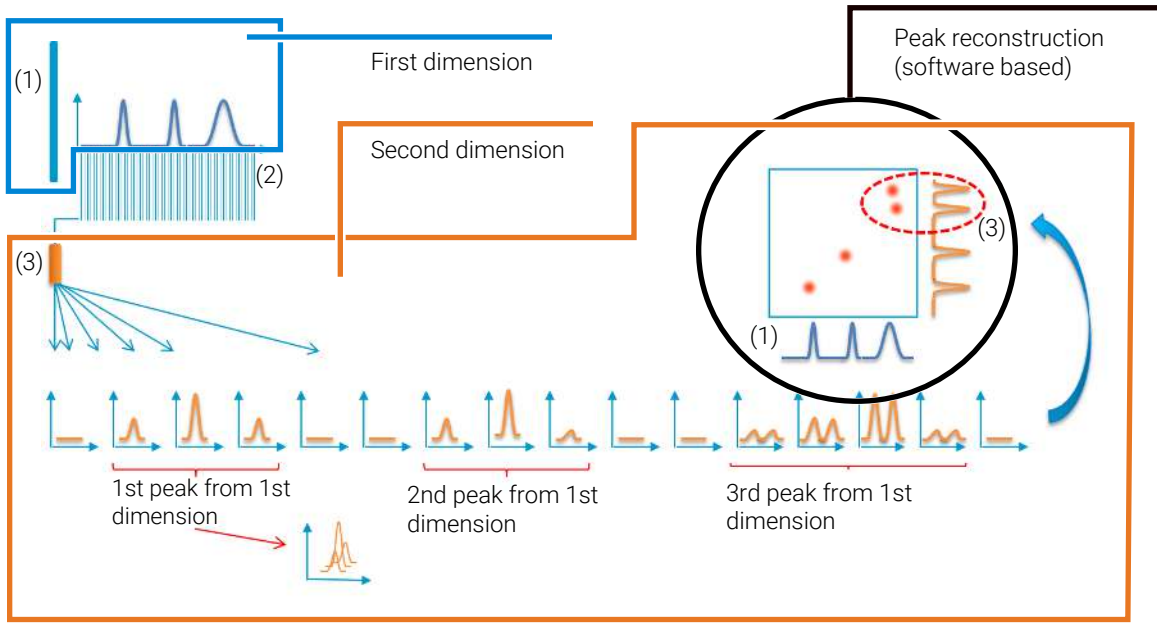


Figure 242: Principle of 2D-LC (example for LCxLC): Effluent of first column (1) is sampled (2) and injected to second column (3). Peaks of second column separation are detected and reconstructed.

	First dimension separation
	Sampling of the first dimension
	Second dimension separation

Successful Mode Combinations

2D-LC separations are the more effective, the more the selectivity mechanisms involved in the two stages differ. Completely different and independ mechanisms are said to be orthogonal. Any correlation between the selectivity mechanisms degrades orthogonality and reduces the efficiency of the 2D-LC system.

Thus, selecting the best combination of stationary and mobile phase is the major issue to improve 2D-LC methods. Table 49 on page 434 summarizes the advantages and disadvantages of combinations of normal phase (NP), reverse phase (RP), ionexchange (IEC) and size exclusion chromatography (SEC) for 2D-LC operation.

Table 49: Mode combinations in 2D-LC (LCxLC)

Combination	Orthogonality	Peak capacity	Application	Comment
RP x RP	[8]	++ [9]	Peptidomics, metabolomics, pharmaceuticals, foods, cosmetics	Miscible solvents, broadest application, fast speed, gradient elution on both dimensions
IEC and RP	+ [10]	-	Proteomics, peptidomics	
SEC and RP	+	-	Polymers, proteomics	
NP and RP	+		Polymers, pharmaceuticals, oils	Solvent incompatibility, limited application
Affinity and RP	+	-	Proteomics	
SEC and NP	+	-	Polymers	
SEC and IEC	+	-	Proteomics	

8 Orthogonality, depends on the column choice or mobile phase choice

9 Very good

10 Good

Solvent Elution Modes

Table 50 on page 437 focuses on the effects of elution modes for ^{2}D separation.

The following elution modes for ^{2}D separation are commonly used:

- Gradient

  A standard gradient of solvent A vs. solvent B for the second dimension separation will be repeated during the complete first dimension separation

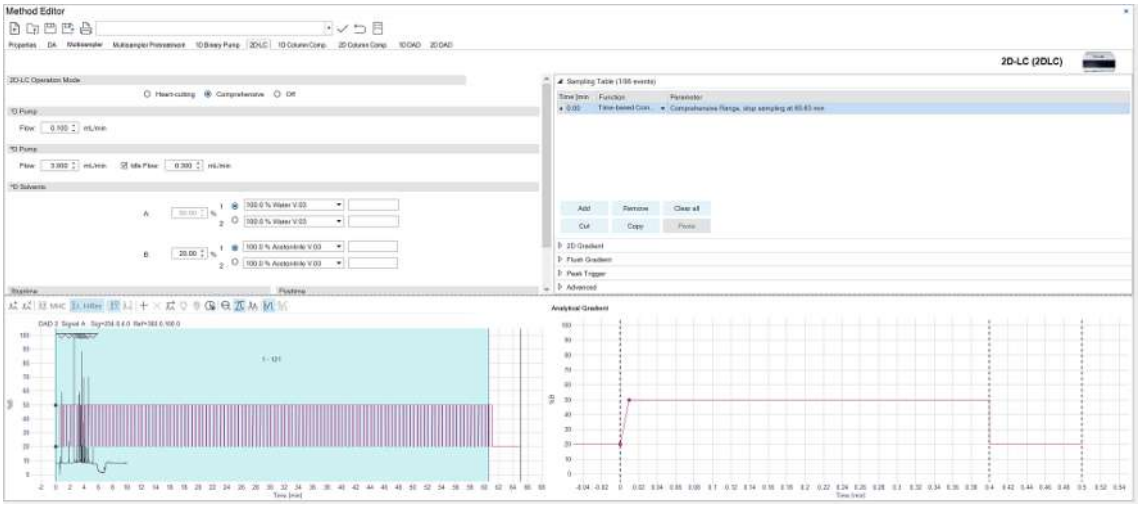


Figure 243: Standard gradient mode

- Shifted Gradient

  From each ^{2}D separation to the next the start-%B and end-%B values of the individual ^{2}D gradients will be increased in a defined way. Additionally, the gradient span can be increased from each ^{2}D gradient to the next.

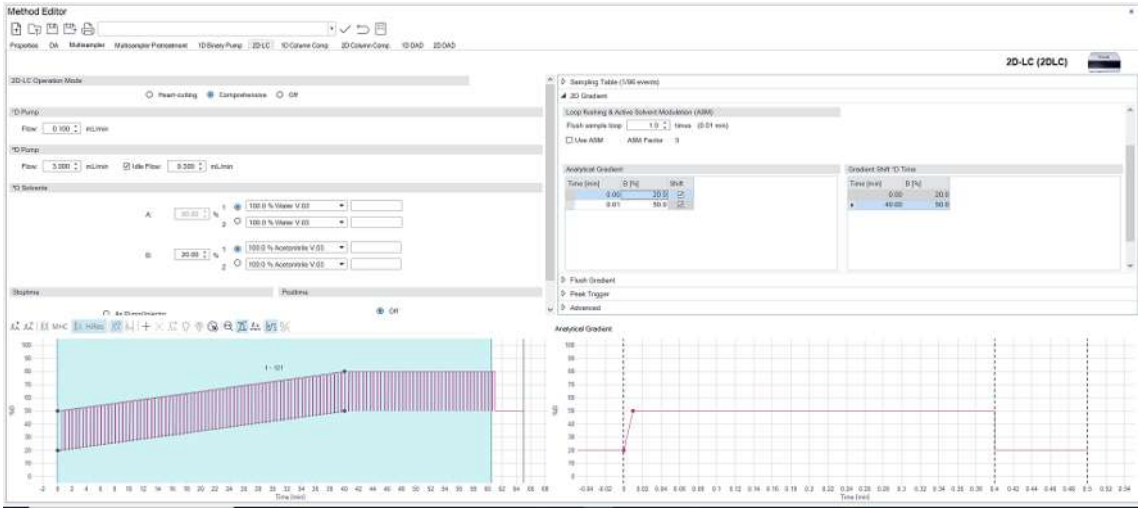


Figure 244: Shifted gradient mode with increase of start-%B

- Isocratic

  All second dimension separations will be carried out in an isocratic mode.

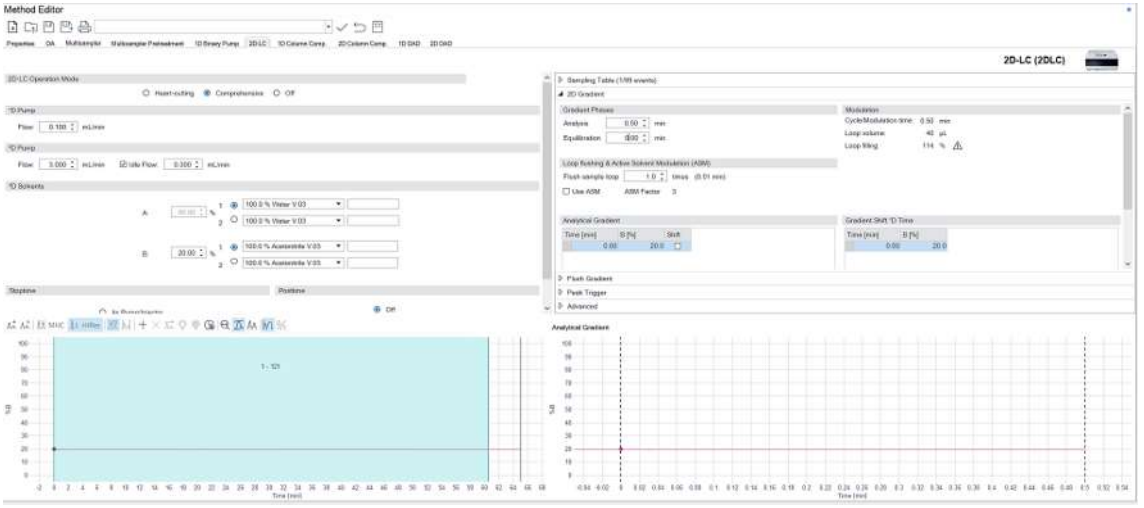


Figure 245: Isocratic mode

- Advancing isocratic

  Nearly isocratic conditions are used in each ^{2}D separation, with slightly increasing solvent strength in each successive run.

The ^{2}D pumping system is fed with a shallow gradient in eluent composition over the course of the ^{2}D separation.

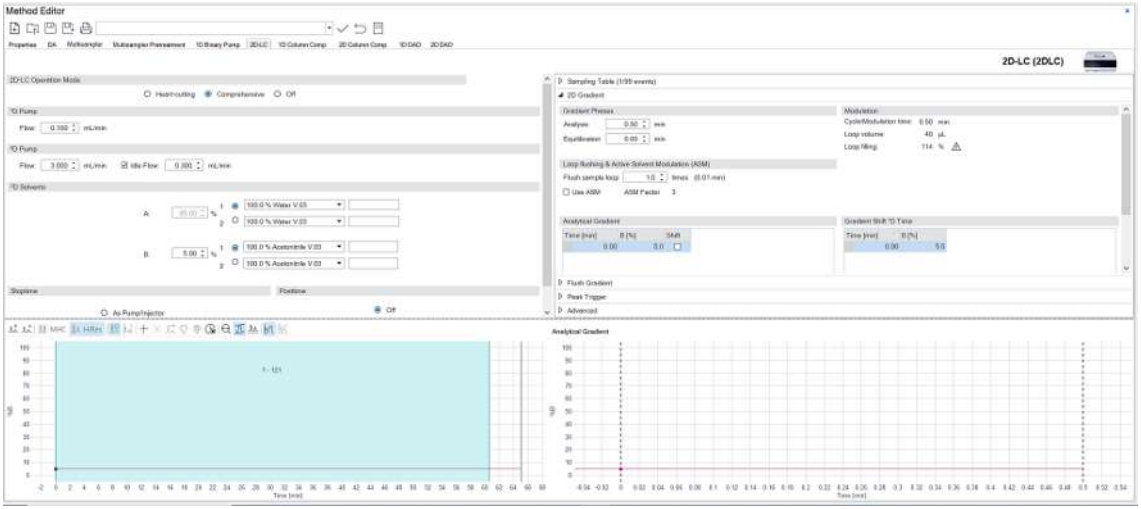


Figure 246: Advancing isocratic mode

Table 50: Different elution modes in the ^{2}D (pros and conts)

Criterion	Gradient/Shifted gradient	Isocratic/Advancing isocratic
Peak capacity	Superior	Inferior
Diversity of samples (complex samples)	Superior	Inferior
Baseline performance (sensitivity	Inferior (baseline drift caused by solvent gradient)	Superior
Pressure stress (column lifetime!)	Inferior (large changes within every 2nd dimension gradient	Superior (no pressure changes with isocratic, gradually changing with advancing isocratic)

All modes are easily available with the Agilent 2D-LC Acquisition software.

Each mode has advantages and disadvantages. No single mode is superior in all applications of 2D-LC.

Effect of shifted gradient elution mode in the ^{2}D

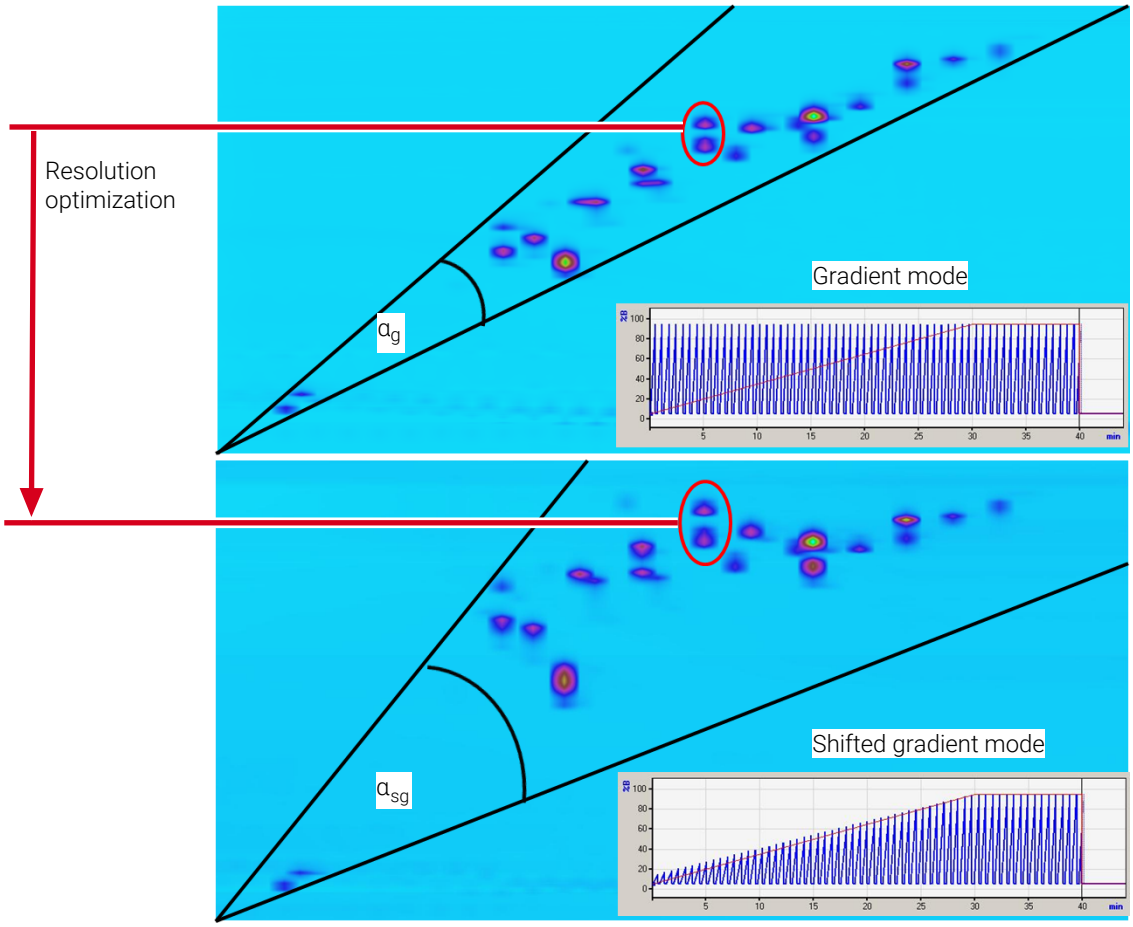


Figure 247: ^{2}D gradient mode compared to isocratic mode and its effect on resolution

α_{sg} as achieved in shifted gradient mode is larger than α_g achieved in standard gradient elution mode. This can lead to an improved peak detection and improved separation.

See *D. Li and O. J. Schmitz "Use of Shift Gradient in the Second Dimension to Improve the Separation Space in Comprehensive Twodimensional Liquid Chromatography" Anal. Bioanal. Chem. 405, 6511-6517 (2013).*

Practical Issues

The table below gives an overview, which practical issues have to be considered in 2D-LC.

Table 51: Practical issues in 2D-LC

Issue	Theoretical base	Comment
Choice of first dimension column diameter	Has impact on trade off between optimum first dimension flow rate and amount of sample injected into the second dimension column for each second column run	True gradient elution in the second dimension separation provides better peak capacity than in isocratic elution. Gradient elution is the best available mechanism for achieving peak focusing.
Ratio of column diameter in the two dimensions	Causes significant analyte dilution effects	
Goals of the analytical method	Chosen parameter depend on what is important in analysis: • separate as many constituents as possible or • focused on resolution and quantitation of a specific constituent	
Selection of the stationary phases and column formats	For RPLC in both dimensions the retentivity of the second dimension column must be much higher than that of the first dimension column required because: • a relatively large volume of the sample will be collected and injected into the second column • to minimize peak broadening the sample should be focused at the inlet of the second column	

Based on theory, in most cases following approaches to achieve best possible 2D-LC should be respected:

- Methodology

  As in Comprehensive 2D-LC is no direct need [11] for UV-detection in the first dimension, other eluents than acetonitrile or methanol are possible. This implies the possibility to use unconventional organic solvents in the first dimension.

11 In case the peak and time triggered operation of the second dimension separation, which is optionally available with the Agilent 1290 Infinity 2D-LC solution, an UV-detector is required between the first dimension column and the modulation valve.

NOTE: Take care when using any unconventional organic solvents that these are still compatible with the used instrumentation. In doubt, refer to the module documentation or call Agilent.

- Instrumentation

  It is important to use very low delay-volume-gradient pumping systems that are able to produce high flow rates to achieve fast second dimension gradients with only little gradient delay - like the Agilent 1290 Infinity LC.

- Columns

  Total orthogonality is difficult to achieve, as there are relatively few combinations sufficiently phase selective.

- Detection methods

  Compared to mass spectrometry DAD based UV detection is faster, cheaper and offers higher reproducibility, thus mass spectrometry offers additional increase in peak capacity by expanding the separation space into the MS-domain. A high sensitivity UV-detector is recommended since a dilution of the first dimension peaks occurs in the second dimension separation – an Agilent 1260 or 1290 Infinity Diode-Array-Detector with 60 mm flow cell is ideal as second dimension detector.

- Data analysis

  2D-LC-data are complex. Use of special software is advisable.

15 Legacy Checkout

This chapter describes the legacy checkout for the 2D-LC Solution in the modes standard heart-cutting, multiple heart-cutting, high-resolution sampling and comprehensive 2D-LC with the driver-based 2D-LC Solution.

Checkout Procedure

The checkout procedure requires 5190-6895 (2D-LC starter sample, 1 x 2 mL) , that contains the following components.

Table 52: Components of 5190-6895

Analyte	CAS#
Atrazine	001912-24-9
Atrazine-desethyl	006190-65-4
Chlorotoluron	015545-48-9
Diuron	000330-54-1
Hexazinone	051235-04-2
Linuron	000330-55-2
Metazachlor	067129-08-2
Methabenzthiazuron	018691-97-9
Metobromuron	003060-89-7
Metoxuron	019937-59-8
Nifedipine	021829-25-4
Nimodipine	066085-59-4
Prometryn	007287-19-6
Sebuthylazine	007286-69-3
Terbuthylazine	005915-41-3
Terbuthylazine-desethyl	030125-63-4

The method parameters described here have been optimized for the following hardware configuration.

Table 53: Hardware configuration for optimized method parameters

	^{1}D	2D-LC	^{2}D
LC	ALS	Universal drives with 2D-LC ASM valve and two MHC valves	
	Pump		Pump

	^{1}D	2D-LC	^{2}D
	MCT		MCT
	UV Detector		UV Detector
LC-MS			High-End mass spectrometers

Prepare the Experiment

Parts required

Qty.	p/n	Description
1	5190-6895	2D-LC starter sample, 1 x 2 mL
1	G2453-85060	Formic Acid-Reagent Grade 5 mL (5 cc)
1	858700-902	RRHD SB-C18, 2.1x100 mm, 1.8 µm, 1200 bar In ^{1}D
1	857768-901	RRHD Bonus-RP, 2.1x50 mm, 1.8 µm, 1200 bar In ^{2}D for Heart Cutting (LC-LC) and High-Resolution (HiRes)
1	959757-302	RRHD Eclipse Plus C18, 3.0x50 mm, 1.8 µm In ^{2}D for Comprehensive 2D-LC (LCXLC)

Parts required

Qty.	p/n	Description
1		Various hardware configurations are possible, see .

Take care that the following solvents for mobile phases are available:

Preparations

1D

- A = water with G2453-85060 (Formic Acid-Reagent Grade 5 mL (5 cc))
- B = methanol

Preparations

2D

- A = water with G2453-85060 (Formic Acid-Reagent Grade 5 mL (5 cc))
- B = acetonitrile

NOTE

Recommended to use legacy setup for the old columns and easy start kit for the new columns.

Preparation of 1.2 mL sample (1:10) for standard LC

1 To prepare 1080 µL dilution solvent, add 216 µL methanol to 864 µL Mobile Phase A. 1080 µL dilution solvent (20 % methanol in mobile phase A) is prepared.

OR: To prepare 3600 µL dilution solvent, add 720 µL methanol to 2880 µL Mobile Phase A. 3600 µL dilution solvent (20 % methanol in mobile phase A) is prepared.

2 To prepare 1.2 mL sample (1:10), add 120 µL 2D-LC starter sample to 1080 µL dilution solvent.

OR: To prepare 4.0 mL sample (1:10), add 400 µL 2D-LC starter sample to 3600 µL dilution solvent.

Dilution of the 2D-LC starter sample in a ratio of 1:100

1 100 µL 2D-LC sample (1:10) + 900 µL dilution solvent = 1000 µL (1:100)

Dilution of the 2D-LC starter sample in a ratio of 1:1000

1 100 µL 2D-LC sample (1:100) + 900 µL dilution solvent = 1000 µL (1:1000)

NOTE For the 2D-LC Addon Software Solution please refer to the User Manual of the Addon Software.

Run the Experiment

Run the Checkout Procedure for Standard Heart-Cutting 2D-LC (LC-LC)

To run the checkout, various hardware configurations are possible, see Table 8 on page 54. Not all options can be shown. As example the Table 53 on page 442 is used here.

The following parameters have been optimized for this standard configuration. Parameters can deviate slightly for your system. Run the experiment and subsequently edit or optimize the method for your setup.

Table 54: Recommended conditions in 1D (HPLC) for SHC 2D-LC

Parameter	Value
	[1]D Column Compartment (MCT)
Column	858700-902
Column temperature	40 °C
Stop time	As pump/No limit
	[1]D Pump
Mobile Phase A	Water + 0.2 % formic acid
Mobile Phase B	Methanol
Flow Rate	0.6 mL/min
Post time	6 min
Mobile Phase Gradient:	20 % B 0.00 min 100 % B 50 min
	Autosampler
Injection Volume	2 µLfor Standard LC 1:10 0.5 µL Positive Mode for LCMS, 1:100 or 1:1000 depending on the used LCMS
Injection Needle Wash	In Flush Port, 10 s, acetonitrile/water (50/50)
Stop time	As pump/No limit
	[1]D Detector (DAD)

Parameter	Value
Diode-array Detector Signal A	254 nm, Bandwidth 4 nm
Reference Wavelength	360 nm
Reference Bandwidth	100 nm
Peak width	20 Hz
Stop time	Stop time As pump/No limit

Table 55: Recommended conditions in 2D (HPLC) for standard heart-cutting

Parameter	Value
	2D-LC Valve
	SHC or MHC with 40 µl sample, Transfer Capillary, ASM Factor No
	[2] **D Column Compartment (MCT)**
Column	857768-901
Column temperature	40 °C
Stop time	As pump/No limit
	[2] **D Pump**
	Heart Cutting (time or peak based)
Mobile Phase A	Water + 0.2 % formic acid
Mobile Phase B	Methanol
Flow Rate	1.0 mL/min
Idle flow	not used
Stop time	40 min (will not automatically prolonged, if peaks in 2D are not work off)
Post time	6 min

Parameter	Value
Sampling Table	Start 4.35 min, minimum 3 cuts required (time based or peak based), Cut Size 4.0

Sampling Table (9/91 events)

Time [min	Function	Parameter
4.35	Time-based Heart...	MHC 1 x 4 s, LoopFill: >300, Prio: -, Default, Index 0, Factor 1 , Multi-Inject False
6.72	Time-based Heart...	MHC 1 x 4 s, LoopFill: >300, Prio: -, Default, Index 0, Factor 1 , Multi-Inject False
10.32	Time-based Heart...	MHC 1 x 4 s, LoopFill: >300, Prio: -, Default, Index 0, Factor 1 , Multi-Inject False
12.39	Time-based Heart...	MHC 1 x 4 s, LoopFill: >300, Prio: -, Default, Index 0, Factor 1 , Multi-Inject False
12.88	Time-based Heart...	MHC 1 x 4 s, LoopFill: >300, Prio: -, Default, Index 0, Factor 1 , Multi-Inject False
13.75	Time-based Heart...	MHC 1 x 4 s, LoopFill: >300, Prio: -, Default, Index 0, Factor 1 , Multi-Inject False
17.05	Time-based Heart...	MHC 1 x 4 s, LoopFill: >300, Prio: -, Default, Index 0, Factor 1 , Multi-Inject False
18.89	Time-based Heart...	MHC 1 x 4 s, LoopFill: >300, Prio: -, Default, Index 0, Factor 1 , Multi-Inject False
24.11	Time-based Heart...	MHC 1 x 4 s, LoopFill: >300, Prio: -, Default, Index 0, Factor 1 , Multi-Inject False

Time-based

Sampling Table (2/98 events)

Time [min	Function	Parameter
3.00	Start Peak-based	MHC 1 x 9 s, Default, Index 0, Exp Time 0 min, RefOnly False, Multi-Inject False
20.00	End Peak-based	

Peak-based
The Cut-Time (SHC) can vary slightly depending on the configuration and the used hardware.

2D Gradient:	Analysis 1.25 min, Equilibration 0.50 min
	Analytical gradient - Shifted Gradient Shift 1D:
	10 % B 0.00 min - 30 % B 20 min 60 % B 1.25 min
Flush gradient	not used
	[2] **D Detector (DAD)**
Diode-array	254 nm, Bandwidth 4 nm
Reference Wavelength	360 nm
Reference Bandwidth	100 nm
Peak width	80 Hz
Stop time	As pump/No limit

Table 56: Recommended conditions in ^{2}D (LC-MS)

Parameter	Value
Ion Source	Atmospheric pressure electrospray (Dual AJS ESI) [6]
Ion Mode	Dual AJS ESI
Ion Polarity	Positive
Storage Mode	Both, Centroid preferred
LCMS Stream	MS
Acquisition Mode	Acquisition Mode MS1 Min Range (m/z) 50 , Max Range (m/z) 500 , Scan Rate (spectra/sec) 3
Instrument Parameters	**Source Parameters**
Gas Temp	250 °C
Gas Flow	11 L/min
Nebulizer	40 psig
SheathGasTemp	350 °C
SheathGasFlow	12 L/min
Scan Segment	1
	Scan Source Parameters
Vcap	3500 V
Nozzle Voltage	300 V
Fragmentor	120
Skimmer1	45
OctopoleRFPeak	750
	ReferenceMasses
Ref Mass Enabled	Enabled
Use Bottle A RefNebulizer	True
Ref Nebulizer	0 psig
	AutoRecalibration
Average Scans	1
Detection Window (ppm)	100 ppm
Min Height	1000 counts
	Reference Masses

Parameter	Value
	Positive
	121.05087300
	922.00979800
	Chromatograms
	Chrom Type Label Offset Y-Range
	TIC TIC 15 10000000
	TIC TIC 15 10000000
Stop Time	As pump/No limit

Table 57: Recommended conditions in ^{2}D (LC-MS) - SQ MS

Parameter	Value
ESI Source Parameter	Similar to the High-end MS parameter
Peak Width	0.06 min
SCAN	100 – 500 m/z in positive mode
Dwell Time	200 ms

1. Load method **Standard Heart-Cutting Checkout** from the 2D-LC data media and modify the settings for your standard heart-cutting configuration.
2. Run the method with 5190-6895 (2D-LC starter sample, 1 x 2 mL) , 1:10 (for only UV Checkout), 1:100 (for LCMS Checkout), or 1:1000 (for LCMS Checkout) diluted with Methanol/Water (20/80; v/v) with 0.1 % formic acid.
3. If necessary, subsequently edit or optimize the method.

Run the Checkout Procedure for Multiple Heart-Cutting (2D-LC)

To run the checkout, various hardware configurations are possible, see Table 8 on page 54. Not all options can be shown. As example the Table 53 on page 442 is used here.

The following parameters have been optimized for this standard configuration. Parameters can deviate slightly for your system. Run the experiment and subsequently edit or optimize the method for your setup.

Table 58: Recommended conditions in 1D (HPLC) for MHC and HiRes 2D-LC

Parameter	Value
	[1]D Column Compartment (MCT)
Column	858700-902
Column temperature	40 °C
Stop time	As pump/No limit
	[1]D Pump
Mobile Phase A	Water + 0.2 % formic acid
Mobile Phase B	Methanol
Flow Rate	0.6 mL/min
Post time	6 min
Mobile Phase Gradient:	20 % B 0.00 min 100 % B 50 min
	Autosampler
Injection Volume	2 µLfor Standard LC 1:10 0.5 µL Positive Mode for LCMS, 1:100 or 1:1000 depending on the used LCMS
Injection Needle Wash	In Flush Port, 10 s, acetonitrile/water (50/50)
Stop time	As pump/No limit
	[1]D Detector (DAD)
Diode-array Detector Signal A	254 nm, Bandwidth 4 nm
Reference Wavelength	360 nm
Reference Bandwidth	100 nm

Parameter	Value
Peak width	20 Hz
Stop time	Stop time As pump/No limit

Table 59: Recommended conditions in 2D (HPLC) for multiple heart-cutting

Parameter	Value
	2D-LC Valve
	MHC with 40 µl sample, Transfer Capillary, ASM Factor No
	^{2}D Column Compartment (MCT)
Column	857768-901
Column temperature	40 °C
Stop time	As pump/No limit
	^{2}D Pump
	Heart Cutting (time or peak based)
Mobile Phase A	Water + 0.2 % formic acid
Mobile Phase B	Acetonitrile
Flow Rate	1 mL/min
Idle flow	not used
Stop time	40 min (will not be automatically prolonged, if peaks in 2D are not work off)
Post time	6 min

Parameter	Value
Sampling Table	Start 4.35 min, minimum 5 cuts required (time based or peak based), Cut Size 4.0

Sampling Table (9/91 events)

Time [min	Function	Parameter
4.35	Time-based Heart...	MHC 1 x 4 s, LoopFill: >300, Prio: -, Default, Index 0, Factor 1 , Multi-Inject False
6.72	Time-based Heart...	MHC 1 x 4 s, LoopFill: >300, Prio: -, Default, Index 0, Factor 1 , Multi-Inject False
10.32	Time-based Heart...	MHC 1 x 4 s, LoopFill: >300, Prio: -, Default, Index 0, Factor 1 , Multi-Inject False
12.39	Time-based Heart...	MHC 1 x 4 s, LoopFill: >300, Prio: -, Default, Index 0, Factor 1 , Multi-Inject False
12.88	Time-based Heart...	MHC 1 x 4 s, LoopFill: >300, Prio: -, Default, Index 0, Factor 1 , Multi-Inject False
13.75	Time-based Heart...	MHC 1 x 4 s, LoopFill: >300, Prio: -, Default, Index 0, Factor 1 , Multi-Inject False
17.05	Time-based Heart...	MHC 1 x 4 s, LoopFill: >300, Prio: -, Default, Index 0, Factor 1 , Multi-Inject False
18.89	Time-based Heart...	MHC 1 x 4 s, LoopFill: >300, Prio: -, Default, Index 0, Factor 1 , Multi-Inject False
24.11	Time-based Heart...	MHC 1 x 4 s, LoopFill: >300, Prio: -, Default, Index 0, Factor 1 , Multi-Inject False

Time-based

Sampling Table (2/98 events)

Time [min	Function	Parameter
3.00	Start Peak-based	MHC 1 x 9 s, Default, Index 0, Exp Time 0 min, RefOnly False, Multi-Inject False
20.00	End Peak-based	

Peak-based
The Cut-Time (MHC) can vary slightly depending on the configuration and the used hardware.

2D Gradient:	Analysis 1.25 min, Equilibration 0.50 min	
	Analytical gradient -	Shifted Gradient Shift 1D:
	10 % B 0.00 min -	30 % B 20 min
	60 % B 1.25 min	
Flush gradient	not used	
	^{2}D Detector (DAD)	
Diode-array	254 nm, Bandwidth 4 nm	
Reference Wavelength	360 nm	
Reference Bandwidth	100 nm	
Peak width	80 Hz	
Stop time	As pump/No limit	

Table 60: Recommended conditions in ^{2}D (LC-MS)

Parameter	Value
Ion Source	Atmospheric pressure electrospray (Dual AJS ESI) [6]
Ion Mode	Dual AJS ESI

Parameter	Value
Ion Polarity	Positive
Storage Mode	Both, Centroid preferred
LCMS Stream	MS
Acquisition Mode	Acquisition Mode MS1 Min Range (m/z) 50 , Max Range (m/z) 500 , Scan Rate (spectra/sec) 3
Instrument Parameters	**Source Parameters**
Gas Temp	250 °C
Gas Flow	11 L/min
Nebulizer	40 psig
SheathGasTemp	350 °C
SheathGasFlow	12 L/min
Scan Segment	1
	Scan Source Parameters
Vcap	3500 V
Nozzle Voltage	300 V
Fragmentor	120
Skimmer1	45
OctopoleRFPeak	750
	ReferenceMasses
Ref Mass Enabled	Enabled
Use Bottle A RefNebulizer	True
Ref Nebulizer	0 psig
	AutoRecalibration
Average Scans	1
Detection Window (ppm)	100 ppm
Min Height	1000 counts
	Reference Masses
	Positive
	121.05087300
	922.00979800

Parameter	Value
	Chromatograms
	Chrom Type Label Offset Y-Range
	TIC TIC 15 10000000
	TIC TIC 15 10000000
Stop Time	As pump/No limit

Table 61: Recommended conditions in ^{2}D (LC-MS) - SQ MS

Parameter	Value
ESI Source Parameter	Similar to the High-end MS parameter
Peak Width	0.06 min
SCAN	100 – 500 m/z in positive mode
Dwell Time	200 ms

1. Load method **Multiple Heart-Cutting Checkout** from the 2D-LC data media and modify the settings for your multiple heart-cutting configuration.
2. Run the method with 5190-6895 (2D-LC starter sample, 1 x 2 mL) , 1:10 (for only UV Checkout), 1:100 (for LCMS Checkout), or 1:1000 (for LCMS Checkout) diluted with Methanol/Water (20/80; v/v) with 0.1 % formic acid.
3. If necessary, subsequently edit or optimize the method.

Run the Checkout Procedure for High Resolution (LC-LC)

To run the checkout, various hardware configurations are possible, see Table 8 on page 54. Not all options can be shown. As example the Table 53 on page 442 is used here.

The following parameters have been optimized for this standard configuration. Parameters can deviate slightly for your system. Run the experiment and subsequently edit or optimize the method for your setup.

Table 62: Recommended conditions in 1D (HPLC) for MHC and HiRes 2D-LC

Parameter	Value
	[1]D Column Compartment (MCT)
Column	858700-902
Column temperature	40 °C
Stop time	As pump/No limit
	[1]D Pump
Mobile Phase A	Water + 0.2 % formic acid
Mobile Phase B	Methanol
Flow Rate	0.6 mL/min
Post time	6 min
Mobile Phase Gradient:	20 % B 0.00 min 100 % B 50 min
	Autosampler
Injection Volume	2 µLfor Standard LC 1:10 0.5 µL Positive Mode for LCMS, 1:100 or 1:1000 depending on the used LCMS
Injection Needle Wash	In Flush Port, 10 s, acetonitrile/water (50/50)
Stop time	As pump/No limit
	[1]D Detector (DAD)
Diode-array Detector Signal A	254 nm, Bandwidth 4 nm
Reference Wavelength	360 nm
Reference Bandwidth	100 nm

Parameter	Value
Peak width	20 Hz
Stop time	Stop time As pump/No limit

Table 63: Recommended conditions in 2D (HPLC) for high resolution

Parameter	Value
	2D-LC Valve
	MHC with 40 µl sample, Transfer Capillary, ASM Factor No
	^{2}D Column Compartment (MCT)
Column	857768-901
Column temperature	40 °C
Stop time	As pump/No limit
	^{2}D Pump
	Heart Cutting (time-based)
Mobile Phase A	Water + 0.2 % formic acid
Mobile Phase B	Acetonitrile
Flow Rate	1 mL/min
Idle flow	not used
Stop time	40 min (will not be automatically prolonged, if peaks in 2D are not work off)
Post time	6 min
Sampling Table	Start 4.28 min, minimum 6 (2*3) HiRes cuts required, Cut Size 3.2 Sampling Table (2/98 events) Time [min / Function / Parameter 4.28 / Time-based Heart... / HiRes 3 x 3.2 s, LoopFill: 80, Prio: -, Default, Index 0, Factor 1 , Multi-Inject False 12.22 / Time-based Heart... / HiRes 9 x 3.2 s, LoopFill: 80, Prio: -, Default, Index 0, Factor 1 , Multi-Inject False HiRes The Cut-Time (HiRes) can vary slightly depending on the configuration and the used hardware.
2D Gradient:	Analysis 1.25 min, Equilibration 0.50 min
	Analytical gradient - Shifted Gradient Shift 1D:
	10 % B 0.00 min - 30 % B 20 min
	60 % B 1.25 min

Parameter	Value
Flush gradient	80 % B 0.00 min + 2 * column void volume corresponds approximately to 0.21 min
	^{2}D Detector (DAD)
Diode-array	254 nm, Bandwidth 4 nm
Reference Wavelength	360 nm
Reference Bandwidth	100 nm
Peak width	80 Hz
Stop time	As pump/No limit

Table 64: Recommended conditions in ^{2}D (LC-MS)

Parameter	Value
Ion Source	Atmospheric pressure electrospray (Dual AJS ESI) [6]
Ion Mode	Dual AJS ESI
Ion Polarity	Positive
Storage Mode	Both, Centroid preferred
LCMS Stream	MS
Acquisition Mode	Acquisition Mode MS1 Min Range (m/z) 50 , Max Range (m/z) 500 , Scan Rate (spectra/sec) 3
Instrument Parameters	**Source Parameters**
Gas Temp	250 °C
Gas Flow	11 L/min
Nebulizer	40 psig
SheathGasTemp	350 °C
SheathGasFlow	12 L/min
Scan Segment	1
	Scan Source Parameters
Vcap	3500 V
Nozzle Voltage	300 V
Fragmentor	120
Skimmer1	45
OctopoleRFPeak	750

Parameter	Value
	ReferenceMasses
Ref Mass Enabled	Enabled
Use Bottle A RefNebulizer	True
Ref Nebulizer	0 psig
	AutoRecalibration
Average Scans	1
Detection Window (ppm)	100 ppm
Min Height	1000 counts
	Reference Masses
	Positive
	121.05087300
	922.00979800
	Chromatograms
	Chrom Type Label Offset Y-Range
	TIC TIC 15 10000000
	TIC TIC 15 10000000
Stop Time	As pump/No limit

Table 65: Recommended conditions in ^{2}D (LC-MS) - SQ MS

Parameter	Value
ESI Source Parameter	Similar to the High-end MS parameter
Peak Width	0.06 min
SCAN	100 – 500 m/z in positive mode
Dwell Time	200 ms

1 Load method High-Resolution Checkout from the 2D-LC data media and modify the settings for your multiple heart cutting configuration.

2 Run the method with 5190-6895 (2D-LC starter sample, 1 x 2 mL) , 1:10 (for only UV Checkout), 1:100 (for LCMS Checkout), or 1:1000 (for LCMS Checkout) diluted with Methanol/Water (20/80; v/v) with 0.1 % formic acid.

3 If necessary, subsequently edit or optimize the method.

Run the Checkout Procedure for Comprehensive (LCXLC)

To run the checkout, various hardware configurations are possible, see Table 8 on page 54. Not all options can be shown. As example the Table 53 on page 442 is used here.

To achieve optimal sensitivity, in comprehensive mode, especially for LC/MS applications, the LC flow is often split prior to the mass spectrometer.

The following parameters have been optimized for this standard configuration. Parameters can deviate slightly for your system. Run the experiment and subsequently edit or optimize the method for your setup.

Table 66: Example for a MS passive splitter setup (ratio 1:2)

Description (PN)	Usage
Tee, Zero 1/16"SS Low dead volume (0100-0969)	T-piece
5067-4659 (5067-4659)	[2]D detector connected to T-piece
5500-1205 (5500-1205)	Inlet of the LCMS source connected to the other end of the T-piece
5500-1206 (5500-1206)	Remaining connection to the T-piece is used as waste capillary

Table 67: Recommended conditions in 1D (HPLC) for comprehensive 2D-LC

Parameter	Value
	[1]D Column Compartment (MCT)
Column	858700-902
Column temperature	40 °C
Stop time	As pump/No limit
	[1]D Pump
Mobile Phase A	Water + 0.2 % formic acid
Mobile Phase B	Methanol
Flow Rate	0.1 mL/min
Stop time	40 min

Parameter	Value
Post time	10 min
Mobile Phase Gradient:	40 % B 0.00 min
	60 % B 34 min
	90 % B 34.5 min
	Autosampler
Injection Volume	2 µLfor Standard LC 0.5 µL Positive Mode for LCMS
Injection Needle Wash	In Flush Port, 10 s, acetonitrile/water (50/50)
Stop time	As pump/No limit
	[1]D Detector (DAD)
Diode-array Detector Signal A	254 nm, Bandwidth 4 nm
Reference Wavelength	360 nm
Reference Bandwidth	100 nm
Peak width	20 Hz
Stop time	Stop time As pump/No limit

Table 68: Recommended conditions in 2D (HPLC) for comprehensive 2D-LC

Parameter	Value
	2D-LC Valve
	MHC with 40 µl sample, Transfer Capillary, ASM Factor No
	[2]D Column Compartment (MCT)
Column	959757-302
Column temperature	40 °C
Stop time	As pump/No limit
	[2]D Pump
	Comprehensive
Mobile Phase A	Water + 0.2 % formic acid
Mobile Phase B	Acetonitrile

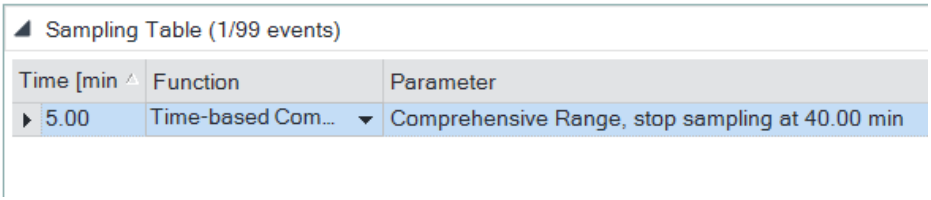
Parameter	Value
Flow Rate	2.5 mL/min
Idle flow	not used
Stop time	ca. 43 min (will not be automatically prolonged, if peaks in 2D are not work off)
Post time	6 min
Sampling Table	Start 5 min, Stop at 40 min  Comprehensive
2D Gradient:	Analysis 0.2 min, Equilibration 0.15 min
	Analytical gradient - Shifted Gradient Shift 1D:
	25 % B 0.00 min 25 % B 5 min 50 % B 40 min
	50 % B 0.2 min 50 % B 5 min 75 % B40 min
	^{2}D Detector (DAD)
Diode-array	254 nm, Bandwidth 4 nm
Reference Wavelength	360 nm
Reference Bandwidth	100 nm
Peak width	80 Hz
Stop time	As pump/No limit

Table 69: Recommended conditions in ^{2}D (LC-MS)

Parameter	Value
Ion Source	Atmospheric pressure electrospray (Dual AJS ESI)
Ion Mode	Dual AJS ESI
Ion polarity	Positive
Storage Mode	Both, Centroid preferred
LCMS Stream	MS
Acquisition Mode	Acquisition Mode MS1 Min Range (m/z) 50 , Max Range (m/z) 500 , Scan Rate (spectra/sec) 3

Parameter	Value
Instrument Parameters	**Source Parameters**
Gas Temp	250 °C
Gas Flow	11 L/min
Nebulizer	40 psig
SheathGasTemp	350 °C
SheathGasFlow	12 L/min
Scan Segment	1
	Scan Source Parameters
Vcap	3500 V
Nozzle Voltage	300 V
Fragmentor	120
Skimmer1	45
OctopoleRFPeak	750
	ReferenceMasses
Ref Mass Enabled	Enabled
Use Bottle A RefNebulizer	True
Ref Nebulizer	0 psig
	AutoRecalibration
Average Scans	1
Detection Window (ppm)	100 ppm
Min Height	1000 counts
	Reference Masses
	Positive
	121.05087300
	922.00979800
	Chromatograms
	Chrom Type Label Offset Y-Range
	TIC TIC 15 10000000
	TIC TIC 15 10000000

Parameter	Value
Stop Time	As pump/No limit
To avoid problems in the LC/MS due to the high flow rate, the effluent from the second dimension column should be split. The recommended split ratio is 1:2	

Table 70: Recommended conditions in ^{2}D (LC-MS) - SQ MS

Parameter	Value
ESI Source Parameter	Similar to the High-end MS parameter
Peak Width	0.06 min
SCAN	100 – 500 m/z in positive mode
Dwell Time	200 ms

1. Load method **Comprehensive Checkout** from the 2D-LC data media and modify the settings for your **Comprehensive** configuration.
2. Run the method with 5190-6895 (2D-LC starter sample, 1 x 2 mL) , 1:10 (for only UV Checkout), 1:100 (for LCMS Checkout), or 1:1000 (for LCMS Checkout) diluted with Methanol/Water (20/80; v/v) with 0.1 % formic acid.
3. If necessary, subsequently edit or optimize the method.

Run the Checkout Procedure for ASM Multiple Heart-Cutting (MHC)

To run the checkout, various hardware configurations are possible, see Table 8 on page 54. Not all options can be shown. As example the Table 53 on page 442 is used here.

The following parameters have been optimized for this standard configuration. Parameters can deviate slightly for your system. Run the experiment and subsequently edit or optimize the method for your setup.

Table 71: Recommended conditions in 1D (HPLC), ASM MHC

Parameter	Value
	[1]D Column Compartment (MCT)
Column	858700-902
Column temperature	40 °C
Stop time	As pump/No limit
	[1]D Pump
Mobile Phase A	Water + 0.2 % formic acid
Mobile Phase B	Methanol
Flow Rate	0.6 mL/min
Stop time	40 min
Post time	6 min
Mobile Phase Gradient:	45 % B 0.00 min
	54 % B 6.00 min
	80 % B 7.00 min
	Autosampler
Injection Volume	2 µLfor Standard LC 0.5 µL Positive Mode for LCMS
Injection Needle Wash	In Flush Port, 10 s, acetonitrile/water (50/50)
Stop time	As pump/No limit
	[1]D Detector (DAD)
Diode-array Detector Signal A	254 nm, Bandwidth 4 nm

Parameter	Value
Reference Wavelength	360 nm
Reference Bandwidth	100 nm
Peak width	20 Hz
Stop time	Stop time As pump/No limit

Table 72: Recommended conditions in 2D (HPLC) for ASM MHC 2D-LC

Parameter	Value
	2D-LC Valve
	MHC with 40 µl sample, Transfer Capillary, ASM Factor 3
	^{2}D Column Compartment (MCT)
Column	857768-901
Column temperature	40 °C
Stop time	As pump/No limit
	^{2}D Pump
Sampling Table	Start 4.35 min, minimum 5 cuts required (time based or peak based), Cut Size 4.0 Sampling Table (9/91 events) Time [min / Function / Parameter 4.35 / Time-based Heart... / MHC 1 x 4 s, LoopFill: >300, Prio: -, Default, Index 0, Factor 1 , Multi-Inject False 6.72 / Time-based Heart... / MHC 1 x 4 s, LoopFill: >300, Prio: -, Default, Index 0, Factor 1 , Multi-Inject False 10.32 / Time-based Heart... / MHC 1 x 4 s, LoopFill: >300, Prio: -, Default, Index 0, Factor 1 , Multi-Inject False 12.39 / Time-based Heart... / MHC 1 x 4 s, LoopFill: >300, Prio: -, Default, Index 0, Factor 1 , Multi-Inject False 12.88 / Time-based Heart... / MHC 1 x 4 s, LoopFill: >300, Prio: -, Default, Index 0, Factor 1 , Multi-Inject False 13.75 / Time-based Heart... / MHC 1 x 4 s, LoopFill: >300, Prio: -, Default, Index 0, Factor 1 , Multi-Inject False 17.05 / Time-based Heart... / MHC 1 x 4 s, LoopFill: >300, Prio: -, Default, Index 0, Factor 1 , Multi-Inject False 18.89 / Time-based Heart... / MHC 1 x 4 s, LoopFill: >300, Prio: -, Default, Index 0, Factor 1 , Multi-Inject False 24.11 / Time-based Heart... / MHC 1 x 4 s, LoopFill: >300, Prio: -, Default, Index 0, Factor 1 , Multi-Inject False Time-based Sampling Table (2/98 events) Time [min / Function / Parameter 3.00 / Start Peak-based / MHC 1 x 9 s, Default, Index 0, Exp Time 0 min, RefOnly False, Multi-Inject False 20.00 / End Peak-based Peak-based The Cut-Time (MHC) can vary slightly depending on the configuration and the used hardware.
Mobile Phase A	Water + 0.2 % formic acid
Mobile Phase B	Acetonitrile

Parameter	Value
Flow Rate	1.0 mL/min
Stop time	ca. 40 min (will not be automatically prolonged, if peaks in 2D are not work off)
Post time	6 min
2D Gradient	Analysis 1.50 min Equilibration 0.50 min Cycle time 2.12 with ASM ON and ASM Factor 3
	Analytical gradient - Shifted Gradient Shift 1D:
	3 % B 0.00 min 3 % B 0.37 min 10 % B 0.38 min 30 % B 6 min 60 % B 1.62
	^{2}D Detector (DAD)
Diode-array	254 nm, Bandwidth 4 nm
Reference Wavelength	360 nm
Reference Bandwidth	100 nm
Peak width	80 Hz
Stop time	As pump/No limit

Table 73: Recommended conditions in ^{2}D (LC-MS)

Parameter	Value
Ion Source	Atmospheric pressure electrospray (Dual AJS ESI) [6]
Ion Mode	Dual AJS ESI
Ion Polarity	Positive
Storage Mode	Both, Centroid preferred
LCMS Stream	MS
Acquisition Mode	Acquisition Mode MS1 Min Range (m/z) 50 , Max Range (m/z) 500 , Scan Rate (spectra/sec) 3
Instrument Parameters	**Source Parameters**
Gas Temp	250 °C
Gas Flow	11 L/min
Nebulizer	40 psig
SheathGasTemp	350 °C
SheathGasFlow	12 L/min
Scan Segment	1

Parameter	Value
	Scan Source Parameters
Vcap	3500 V
Nozzle Voltage	300 V
Fragmentor	120
Skimmer1	45
OctopoleRFPeak	750
	ReferenceMasses
Ref Mass Enabled	Enabled
Use Bottle A RefNebulizer	True
Ref Nebulizer	0 psig
	AutoRecalibration
Average Scans	1
Detection Window (ppm)	100 ppm
Min Height	1000 counts
	Reference Masses
	Positive
	121.05087300
	922.00979800
	Chromatograms
	Chrom Type Label Offset Y-Range
	TIC TIC 15 10000000
	TIC TIC 15 10000000
Stop Time	As pump/No limit

Table 74: Recommended conditions in ^{2}D (LC-MS) - SQ MS

Parameter	Value
ESI Source Parameter	Similar to the High-end MS parameter
Peak Width	0.06 min

Parameter	Value
SCAN	100 – 500 m/z in positive mode
Dwell Time	200 ms

NOTE

Adjust the ASM split ratio
To optimize the ASM split ratio of the method either for highest resolution (strong dilution), or lowest cycle time (weak dilution), different ASM capillaries are available.
The checkout method uses ASM factor 3, see Understanding the ASM factor on page 41.

1 Load method ASM Multiple Heart-Cutting Checkout from the 2D-LC data media and modify the settings for your multiple heart-cutting configuration.

2 Run the method with 5190-6895 (2D-LC starter sample, 1 x 2 mL) , 1:10 (for only UV Checkout), 1:100 (for LCMS Checkout), or 1:1000 (for LCMS Checkout) diluted with Methanol/Water (20/80; v/v) with 0.1 % formic acid.

3 If necessary, subsequently edit or optimize the method.

Run the Checkout Procedure for ASM Comprehensive (ASM OFFASM OFF)

To run the checkout, various hardware configurations are possible, see Table 8 on page 54. Not all options can be shown. As example Table 53 on page 442 is used here.

To achieve optimal sensitivity, in comprehensive mode, especially for LC/MS applications, the LC flow is often split prior to the mass spectrometer. The following parameters have been optimized for this standard configuration. Parameters can deviate slightly for your system. Run the experiment with ASM OFF and subsequently edit or optimize the method for your setup.

Table 75: Example for a MS passive splitter setup (ratio 1:2)

Description (PN)	Usage
Tee, Zero 1/16"SS Low dead volume (0100-0969)	T-piece
5067-4659 (5067-4659)	[2]D detector connected to T-piece
5500-1205 (5500-1205)	Inlet of the LCMS source connected to the other end of the T-piece
5500-1206 (5500-1206)	Remaining connection to the T-piece is used as waste capillary

Table 76: Recommended conditions in 1D (HPLC), ASM comprehensive

Parameter	Value
	[1]D Column Compartment (MCT)
Column	858700-902
Column temperature	40 °C
Stop time	As pump/No limit
	[1]D Pump
Mobile Phase A	Water + 0.2 % formic acid
Mobile Phase B	Methanol
Flow Rate	0.1 mL/min

Parameter	Value
Stop time	40 min
Post time	6 min
Mobile Phase Gradient:	20 % B 0.00 min
	100 % B 50 min
	80 % B 7.00 min
	Autosampler
Injection Volume	2 µLfor Standard LC 0.5 µL Positive Mode for LCMS
Injection Needle Wash	In Flush Port, 10 s, acetonitrile/water (50/50)
Stop time	As pump/No limit
	[1]D Detector (DAD)
Diode-array Detector Signal A	254 nm, Bandwidth 4 nm
Reference Wavelength	360 nm
Reference Bandwidth	100 nm
Peak width	20 Hz
Stop time	Stop time As pump/No limit

Table 77: Recommended conditions in 2D (HPLC) for ASM comprehensive 2D-LC

Parameter	Value
	2D-LC Valve
	MHC with 40 µl sample, Transfer Capillary, ASM Factor No
	[2]D Column Compartment (MCT)
Column	959757-302
Column temperature	40 °C
Stop time	As pump/No limit
	[2]D Pump

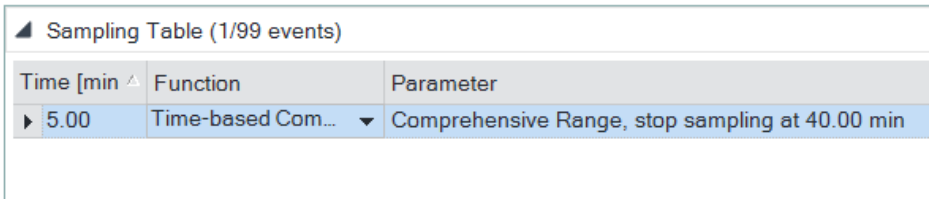
Parameter	Value
	Comprehensive  Comprehensive
Mobile Phase A	Water + 0.2 % formic acid
Mobile Phase B	Methanol
Flow Rate	2.5 mL/min
Stop time	40 min (will not automatically prolonged, if peaks in 2D are not work off)
Post time	6 min
2D gradient	Analysis 0.2 min Equilbration 0.1 min **ASM Off**
	Analytical gradient - shifted gradient shift 1D
	25 % B 0.00 min 25 % B 5 min 50 % B 40 min 50 % B 0.20 min 50 % B 5 min 75 % B 40 min
	^{2}D Detector (DAD)
Diode-array	254 nm, Bandwidth 4 nm
Reference Wavelength	360 nm
Reference Bandwidth	100 nm
Peak width	80 Hz
Stop time	As pump/No limit

Table 78: Recommended conditions in ^{2}D (LC-MS)

Parameter	Value
Ion Source	Atmospheric pressure electrospray (Dual AJS ESI)
Ion Mode	Dual AJS ESI
Ion polarity	Positive
Storage Mode	Both, Centroid preferred
LCMS Stream	MS

Parameter	Value
Acquisition Mode	Acquisition Mode MS1 Min Range (m/z) 50 , Max Range (m/z) 500 , Scan Rate (spectra/sec) 3
Instrument Parameters	**Source Parameters**
Gas Temp	250 °C
Gas Flow	11 L/min
Nebulizer	40 psig
SheathGasTemp	350 °C
SheathGasFlow	12 L/min
Scan Segment	1
	Scan Source Parameters
Vcap	3500 V
Nozzle Voltage	300 V
Fragmentor	120
Skimmer1	45
OctopoleRFPeak	750
	ReferenceMasses
Ref Mass Enabled	Enabled
Use Bottle A RefNebulizer	True
Ref Nebulizer	0 psig
	AutoRecalibration
Average Scans	1
Detection Window (ppm)	100 ppm
Min Height	1000 counts
	Reference Masses
	Positive
	121.05087300
	922.00979800
	Chromatograms
	Chrom Type Label Offset Y-Range
	TIC TIC 15 10000000

Parameter	Value
	TIC TIC 15 10000000
Stop Time	As pump/No limit
To avoid problems in the LC/MS due to the high flow rate, the effluent from the second dimension column should be split. The recommended split ratio is 1:2	

Table 79: Recommended conditions in ^{2}D (LC-MS) - SQ MS

Parameter	Value
ESI Source Parameter	Similar to the High-end MS parameter
Peak Width	0.06 min
SCAN	100 – 500 m/z in positive mode
Dwell Time	200 ms

NOTE Active ASM for comprehensive applications is not recommended as the wear of the valve increases dramatically due to the many switching cycles.

1. Load method **ASM Comprehensive Checkout** from the 2D-LC data media and modify the settings for your configuration.
2. Run the method with 5190-6895 (2D-LC starter sample, 1 x 2 mL) , 1:10 (for only UV Checkout), 1:100 (for LCMS Checkout), or 1:1000 (for LCMS Checkout) diluted with Methanol/Water (20/80; v/v) with 0.1 % formic acid.
3. If necessary, subsequently edit or optimize the method.

16 Appendix

This chapter provides additional information on safety, legal and web.

General Safety Information

The following general safety precautions must be observed during all phases of operation, service, and repair of this instrument. Failure to comply with these precautions or with specific warnings elsewhere in this manual violates safety standards of design, manufacture, and intended use of the instrument. Agilent Technologies assumes no liability for the customer's failure to comply with these requirements.

WARNING

Ensure the proper usage of the equipment.

The protection provided by the equipment may be impaired.

- **The operator of this instrument is advised to use the equipment in a manner as specified in this manual.**

Safety Standards

This is a Safety Class I instrument (provided with terminal for protective earthing) and has been manufactured and tested according to international safety standards.

General

Do not use this product in any manner not specified by the manufacturer. The protective features of this product may be impaired if it is used in a manner not specified in the operation instructions.

Before Applying Power

WARNING Wrong voltage range, frequency or cabling

Personal injury or damage to the instrument

- Verify that the voltage range and frequency of your power distribution matches to the power specification of the individual instrument.
- Never use cables other than the ones supplied by Agilent Technologies to ensure proper functionality and compliance with safety or EMC regulations.
- Make all connections to the unit before applying power.

WARNING Use of unsupplied cables

Using cables not supplied by Agilent Technologies can lead to damage of the electronic components or personal injury.

- Never use cables other than the ones supplied by Agilent Technologies to ensure proper functionality and compliance with safety or EMC regulations.

NOTE Note the instrument's external markings described under Safety Symbols on page 482.

Ground the Instrument

WARNING Missing electrical ground

Electrical shock

- If your product is provided with a grounding type power plug, the instrument chassis and cover must be connected to an electrical ground to minimize shock hazard.
- The ground pin must be firmly connected to an electrical ground (safety ground) terminal at the power outlet. Any interruption of the protective (grounding) conductor or disconnection of the protective earth terminal will cause a potential shock hazard that could result in personal injury.

Do Not Operate in an Explosive Atmosphere

WARNING

Presence of flammable gases or fumes

Explosion hazard

- **Do not operate the instrument in the presence of flammable gases or fumes.**

Do Not Remove the Instrument Cover

WARNING

Instrument covers removed

Electrical shock

- **Do Not Remove the Instrument Cover**
- **Only Agilent authorized personnel are allowed to remove instrument covers. Always disconnect the power cables and any external circuits before removing the instrument cover.**

Do Not Modify the Instrument

Do not install substitute parts or perform any unauthorized modification to the product. Return the product to an Agilent Sales and Service Office for service and repair to ensure that safety features are maintained.

In Case of Damage

WARNING

Damage to the module

Personal injury (for example electrical shock, intoxication)

- **Instruments that appear damaged or defective should be made inoperative and secured against unintended operation until they can be repaired by qualified service personnel.**

Solvent Information

WARNING **Toxic, flammable and hazardous solvents, samples and reagents**

The handling of solvents, samples and reagents can hold health and safety risks.

- **When working with these substances observe appropriate safety procedures (for example by wearing goggles, safety gloves and protective clothing) as described in the material handling and safety data sheet supplied by the vendor, and follow good laboratory practice.**
- **Do not use solvents with an auto-ignition temperature below 200 °C (392 °F). Do not use solvents with a boiling point below 56 °C (133 °F).**
- **Avoid high vapor concentrations. Keep the solvent temperature at least 40 °C (72 °F) below the boiling point of the solvent used. This includes the solvent temperature in the sample compartment. For the solvents methanol and ethanol keep the solvent temperature at least 25 °C (45 °F) below the boiling point.**
- **Do not operate the instrument in an explosive atmosphere.**
- **Do not use solvents of ignition Class IIC according IEC 60079-20-1 (for example, carbon disulfide).**
- **Reduce the volume of substances to the minimum required for the analysis.**
- **Never exceed the maximum permissible volume of solvents (8 L) in the solvent cabinet. Do not use bottles that exceed the maximum permissible volume as specified in the usage guideline for solvent cabinet.**
- **Ground the waste container.**
- **Regularly check the filling level of the waste container. The residual free volume in the waste container must be large enough to collect the waste liquid.**
- **To achieve maximal safety, regularly check the tubing for correct installation.**

NOTE For details, see the usage guideline for the solvent cabinet. A printed copy of the guideline has been shipped with the solvent cabinet, electronic copies are available in the Agilent Information Center or via the Internet.

Recommendations on the Use of Solvents

Observe the following recommendations on the use of solvents.

- Brown glass ware can avoid growth of algae.
- Follow the recommendations for avoiding the growth of algae, see the pump manuals.
- Small particles can permanently block capillaries and valves. Therefore, always filter solvents through 0.22 µm filters.
- Avoid or minimize the use of solvents that may corrode parts in the flow path. Consider specifications for the pH range given for different materials such as flow cells, valve materials etc. and recommendations in subsequent sections.
- Avoid the use of the following steel-corrosive solvents:
  - solutions of alkali halides and their respective acids (for example, lithium iodide, potassium chloride, and so on),
  - high concentrations of inorganic acids like sulfuric acid and nitric acid, especially at higher temperatures (if your chromatography method allows, replace by phosphoric acid or phosphate buffer which are less corrosive against stainless steel),
  - halogenated solvents or mixtures which form radicals and/or acids, for example:

    $2CHCl_3 + O_2 \rightarrow 2COCl_2 + 2HCl$

    This reaction, in which stainless steel probably acts as a catalyst, occurs quickly with dried chloroform if the drying process removes the stabilizing alcohol,
  - chromatographic grade ethers, which can contain peroxides (for example, THF, dioxane, diisopropyl ether) should be filtered through dry aluminium oxide which adsorbs the peroxides,
  - solvents containing strong complexing agents (e.g. EDTA),
  - mixtures of carbon tetrachloride with 2-propanol or THF.
- Avoid the use of dimethyl formamide (DMF). Polyvinylidene fluoride (PVDF), which is used in leak sensors, is not resistant to DMF.

Safety Symbols

Table 80: Symbols

	The apparatus is marked with this symbol when the user shall refer to the instruction manual in order to protect risk of harm to the operator and to protect the apparatus against damage.
	Indicates dangerous voltages.
	Indicates a protected ground terminal.
	The apparatus is marked with this symbol when hot surfaces are available and the user should not touch it when heated up.
	Indicates flammable material used. Consult the Agilent Information Center / User Manual before attempting to install or service this equipment. Follow all safety precautions.
	Confirms that a manufactured product complies with all applicable European Community directives. The European Declaration of Conformity is available at: http://regulations.corporate.agilent.com/DoC/search.htm
	Manufacturing date.
	Product Number
	Serial Number
	Power symbol indicates On/Off. The apparatus is not completely disconnected from the mains supply when the on/off switch is in the Off position
	Pacemaker Magnets could affect the functioning of pacemakers and implanted heart defibrillators. A pacemaker could switch into test mode and cause illness. A heart defibrillator may stop working. If you wear these devices keep at least 55 mm distance to magnets. Warn others who wear these devices from getting too close to magnets.

	Magnetic field Magnets produce a far-reaching, strong magnetic field. They could damage TVs and laptops, computer hard drives, credit and ATM cards, data storage media, mechanical watches, hearing aids and speakers. Keep magnets at least 25 mm away from devices and objects that could be damaged by strong magnetic fields.
	Indicates a pinching or crushing hazard
	Indicates a piercing or cutting hazard.

WARNING

A WARNING

alerts you to situations that could cause physical injury or death.

- **Do not proceed beyond a warning until you have fully understood and met the indicated conditions.**

CAUTION

A CAUTION

alerts you to situations that could cause loss of data, or damage of equipment.

- Do not proceed beyond a caution until you have fully understood and met the indicated conditions.

Material Information

This section provides detailed information about materials used in the HPLC system and general information about solvent/material compatibility.

General Information About Solvent/Material Compatibility

Materials in the flow path are carefully selected based on Agilent's experiences in developing highest-quality instruments for HPLC analysis over several decades. These materials exhibit excellent robustness under typical HPLC conditions. For any special condition, please consult the material information section or contact Agilent.

Disclaimer

Subsequent data was collected from external resources and is meant as a reference. Agilent cannot guarantee the correctness and completeness of such information. Data is based on compatibility libraries, which are not specific for estimating the long-term life time under specific but highly variable conditions of UHPLC systems, solvents, solvent mixtures, and samples. Information also cannot be generalized due to catalytic effects of impurities like metal ions, complexing agents, oxygen etc. Apart from pure chemical corrosion, other effects like electro corrosion, electrostatic charging (especially for nonconductive organic solvents), swelling of polymer parts etc. need to be considered. Most data available refers to room temperature (typically 20 – 25 °C, 68 – 77 °F). If corrosion is possible, it usually accelerates at higher temperatures. If in doubt, please consult technical literature on chemical compatibility of materials.

MP35N

MP35N is a nonmagnetic, nickel-cobalt-chromium-molybdenum alloy demonstrating excellent corrosion resistance (for example, against nitric and sulfuric acids, sodium hydroxide, and seawater) over a wide range of concentrations and temperatures. In addition, this alloy shows exceptional

resistance to high-temperature oxidation. Due to excellent chemical resistance and toughness, the alloy is used in diverse applications: dental products, medical devices, nonmagnetic electrical components, chemical and food processing equipment, marine equipment. Treatment of MP35N alloy samples with 10 % NaCl in HCl (pH 2.0) does not reveal any detectable corrosion. MP35N also demonstrates excellent corrosion resistance in a humid environment. Although the influence of a broad variety of solvents and conditions has been tested, users should keep in mind that multiple factors can affect corrosion rates, such as temperature, concentration, pH, impurities, stress, surface finish, and dissimilar metal contacts.

Polyphenylene Sulfide (PPS)

Polyphenylene sulfide has outstanding stability even at elevated temperatures. It is resistant to dilute solutions of most inorganic acids, but it can be attacked by some organic compounds and oxidizing reagents. Nonoxidizing inorganic acids, such as sulfuric acid and phosphoric acid, have little effect on polyphenylene sulfide, but at high concentrations and temperatures, they can still cause material damage. Nonoxidizing organic chemicals generally have little effect on polyphenylene sulfide stability, but amines, aromatic compounds, and halogenated compounds may cause some swelling and softening over extended periods of time at elevated temperatures. Strong oxidizing acids, such as nitric acid (> 0.1 %), hydrogen halides (> 0.1 %), peroxy acids (> 1 %), or chlorosulfuric acid degrade polyphenylene sulfide. It is not recommended to use polyphenylene sulfide with oxidizing material, such as sodium hypochlorite and hydrogen peroxide. However, under mild environmental conditions, at low concentrations and for short exposure times, polyphenylene sulfide can withstand these chemicals, for example, as ingredients of common disinfectant solutions.

PEEK

PEEK (Polyether-Ether Ketones) combines excellent properties regarding biocompatibility, chemical resistance, mechanical and thermal stability. PEEK is therefore the material of choice for UHPLC and biochemical instrumentation.

It is stable in the specified pH range (for the Bio-Inert LC system: pH 1 – 13 , see bio-inert module manuals for details), and inert to many common solvents.

There are still some known incompatibilities with chemicals such as chloroform, methylene chloride, THF, DMSO, strong acids (nitric acid > 10 %, sulfuric acid > 10 %, sulfonic acids, trichloroacetic acid), halogens or aqueous halogen solutions, phenol and derivatives (cresols, salicylic acid, and so on).

When used above room temperature, PEEK is sensitive to bases and various organic solvents, which can cause it to swell. Under such conditions, normal PEEK capillaries are sensitive to high pressure. Therefore, Agilent uses stainless steel clad PEEK capillaries in bio-inert systems. The use of stainless steel clad PEEK capillaries keeps the flow path free of steel and ensures pressure stability up to 600 bar. If in doubt, consult the available literature about the chemical compatibility of PEEK.

Polyimide

Agilent uses semi-crystalline polyimide for rotor seals in valves and needle seats in autosamplers. One supplier of polyimide is DuPont, which brands polyimide as Vespel, which is also used by Agilent.

Polyimide is stable in a pH range between 1 and 10 and in most organic solvents. It is incompatible with concentrated mineral acids (e.g. sulphuric acid), glacial acetic acid, DMSO and THF. It is also degraded by nucleophilic substances like ammonia (e.g. ammonium salts in basic conditions) or acetates.

Polyethylene (PE)

Agilent uses UHMW (ultra-high molecular weight)-PE/PTFE blends for yellow piston and wash seals, which are used in 1290 pumps, the G7104C and for normal phase applications in 1260 pumps.

Polyethylene has a good stability for most common inorganic solvents including acids and bases in a pH range of 1 to 12.5 . It is compatible with many organic solvents used in chromatographic systems like methanol, acetonitrile and isopropanol. It has limited stability with aliphatic, aromatic and halogenated hydrocarbons, THF, phenol and derivatives, concentrated acids and bases. For normal phase applications, the maximum pressure should be limited to 200 bar.

Tantalum (Ta)

Tantalum is inert to most common HPLC solvents and almost all acids except fluoric acid and acids with free sulfur trioxide. It can be corroded by strong bases (e.g. hydroxide solutions > 10 %, diethylamine). It is not recommended for the use with fluoric acid and fluorides.

Stainless Steel (SST)

Stainless steel is inert against many common solvents. It is stable in the presence of acids and bases in a pH range of 1 to 12.5 . It can be corroded by acids below pH 2.3 . It can also corrode in following solvents:

- Solutions of alkali halides, their respective acids (for example, lithium iodide, potassium chloride) and aqueous solutions of halogens.
- High concentrations of inorganic acids like nitric acid, sulfuric acid, and organic solvents especially at higher temperatures (replace, if your chromatography method allows, by phosphoric acid or phosphate buffer, which are less corrosive against stainless steel).
- Halogenated solvents or mixtures, which form radicals and/or acids, for example:

  $2\ CHCl_3 + O_2 \rightarrow 2\ COCl_2 + 2\ HCl$

  This reaction, in which stainless steel probably acts as a catalyst, occurs quickly with dried chloroform if the drying process removes the stabilizing alcohol.
- Chromatographic grade ethers, which can contain peroxides (for example, THF, dioxane, diisopropyl ether). Such ethers should be filtered through dry aluminum oxide, which adsorbs the peroxides.
- Solutions of organic acids (acetic acid, formic acid, and so on) in organic solvents. For example, a 1 % solution of acetic acid in methanol will attack steel.
- Solutions containing strong complexing agents (for example, EDTA, ethylenediaminetetraacetic acid).
- Mixtures of carbon tetrachloride with isopropanol or THF.

Titanium (Ti)

Titanium is highly resistant to oxidizing acids (for example, nitric, perchloric and hypochlorous acid) over a wide range of concentrations and temperatures. This is due to a thin oxide layer on the surface, which is stabilized by oxidizing compounds. Non-oxidizing acids (for example, hydrochloric, sulfuric and phosphoric acid) can cause slight corrosion, which increases with acid concentration and temperature. For example, the corrosion rate with 3 % HCl (about pH 0.1) at room temperature is about 13 µm/year. At room temperature, titanium is resistant to concentrations of about 5 % sulfuric acid (about pH 0.3). Addition of nitric acid to hydrochloric or sulfuric acids significantly reduces corrosion rates. Titanium is sensitive to acidic metal chlorides like $FeCl_3$ or $CuCl_2$.

Titanium is subject to corrosion in anhydrous methanol, which can be avoided by adding a small amount of water (about 3 %). Slight corrosion is possible with ammonia > 10 %.

Diamond-Like Carbon (DLC)

Diamond-Like Carbon is inert to almost all common acids, bases, and solvents. There are no documented incompatibilities for HPLC applications.

Fused Silica and Quartz (SiO_2)

Fused silica is used in Max Light Cartridges. Quartz is used for classical flow cell windows. It is inert against all common solvents and acids except hydrofluoric acid and acidic solvents containing fluorides. It is corroded by strong bases and should not be used above pH 12 at room temperature. The corrosion of flow cell windows can negatively affect measurement results. For a pH greater than 12, the use of flow cells with sapphire windows is recommended.

Gold

Gold is inert to all common HPLC solvents, acids, and bases within the specified pH range. It can be corroded by complexing cyanides and concentrated acids like aqua regia.

Zirconium Oxide (ZrO_2)

Zirconium Oxide is inert to almost all common acids, bases, and solvents. There are no documented incompatibilities for HPLC applications.

Platinum/Iridium

Platinum/Iridium is inert to almost all common acids, bases, and solvents. There are no documented incompatibilities for HPLC applications.

Fluorinated Polymers (PTFE, PFA, FEP, FFKM, PVDF)

Fluorinated polymers like PTFE (polytetrafluorethylene), PFA (perfluoroalkoxy), and FEP (fluorinated ethylene propylene) are inert to almost all common acids, bases, and solvents. FFKM is perfluorinated rubber, which is also resistant to most chemicals. As an elastomer, it may swell in some organic solvents like halogenated hydrocarbons.

TFE/PDD copolymer tubings, which are used in all Agilent degassers except G1322A/G7122A, are not compatible with fluorinated solvents like Freon, Fluorinert, or Vertrel. They have limited life time in the presence of hexafluoroisopropanol (HFIP). To ensure the longest possible life with HFIP, it is best to dedicate a particular chamber to this solvent, not to switch solvents, and not to let dry out the chamber. For optimizing the life of the pressure sensor, do not leave HFIP in the chamber when the unit is off.

The tubing of the leak sensor is made of PVDF (polyvinylidene fluoride), which is incompatible with the solvent DMF (dimethylformamide).

Sapphire, Ruby, and Al_2O_3-Based Ceramics

Sapphire, ruby, and ceramics based on aluminum oxide Al_2O_3 are inert to almost all common acids, bases, and solvents. There are no documented incompatibilities for HPLC applications.

At-a-Glance Details About Agilent Capillaries

The following section provides useful information about Agilent capillaries and its characteristics.

Syntax for capillary description

Type - Material - Capillary dimensions - Fitting Left/Fitting right

Table 81: Example for a capillary description

Code provided with the part	Meaing of the code
Color code: 	Material of the product is MP35N, the inner diameter is 0.20 or 0.25 mm
Capillary	The part is a connection capillary
MP35N	Material of the part is MP35N
0.25 x 80 mm	The part has an inner diameter of 0.25 mm and a length of 80 mm
SI/SI	Left fitting: Swagelok + 1.6 mm Port id, Intermediate Right fitting: Swagelok + 1.6 mm Port id, Intermediate

To get an overview of the code in use, see

- Color: Table 82 on page 491
- Type: Table 83 on page 491
- Material: Table 84 on page 492
- Dimension: Table 85 on page 492
- Fittings: Table 86 on page 493

Color Coding Guide

Table 82: Color-coding key for Agilent capillary tubing

Internal diameter in mm		Color code
0.015		Orange
0.025		Yellow
0.05		Beige
0.075		Black
0.075	MP35N	Black with orange stripe
0.1		Purple
0.12		Red
0.12	MP35N	Red with orange stripe
0.17		Green
0.17	MP35N	Green with orange stripe
0.20 /0.25		Blue
0.20 /0.25	MP35N	Blue with orange stripe
0.3		Grey
0.50		Bone White

NOTE

As you move to smaller-volume, high efficiency columns, you'll want to use narrow id tubing, as opposed to the wider id tubing used for conventional HPLC instruments.

Abbreviation Guide for Type

Table 83: Type (gives some indication on the primary function, like a loop or a connection capillary)

Key	Description
Capillary	Connection capillaries
Loop	Loop capillaries
Seat	Autosampler needle seats

Key	Description
Tube	Tubing
Heat exchanger	Heat exchanger

Abbreviation Guide for Material

Table 84: Material (indicates which raw material is used for the capillary)

Key	Description
ST	Stainless steel
Ti	Titanium
PK	PEEK
FS/PK	PEEK-coated fused silica [12]
PK/ST	Stainless steel-coated PEEK [13]
PFFE	PTFE
FS	Fused silica
MP35N	Nickel-cobalt-chromium-molybdenium alloy

Abbreviation Guide for Capillary Dimensions

Table 85: Capillary dimensions (indicates inner diameter (id), length, and volume of the capillary)

Description
id (mm) x Length (mm)
Volume (µL)

12 Fused silica in contact with solvent

13 Stainless steel-coated PEEK

Abbreviation Guide for Fitting Left/Fitting Right

Table 86: Fitting left/fitting right (indicates which fitting is used on both ends of the capillary)

Key	Description
W	Swagelok + 0.8 mm Port id
S	Swagelok + 1.6 mm Port id
M	Metric M4 + 0.8 mm Port id
E	Metric M3 + 1.6 mm Port id
U	Swagelok union
L	Long
X	Extra long
H	Long head
G	Small head SW 4
N	Small head SW 5
F	Finger-tight
V	1200 bar
B	Bio
P	PEEK
I	Intermediate

Waste Electrical and Electronic Equipment (WEEE) Directive

This product complies with the European WEEE Directive marking requirements. The affixed label indicates that you must not discard this electrical/electronic product in domestic household waste.

NOTE

Do not dispose of in domestic household waste
To return unwanted products, contact your local Agilent office, or see https://www.agilent.com for more information.

Radio Interference

Cables supplied by Agilent Technologies are screened to provide optimized protection against radio interference. All cables are in compliance with safety or EMC regulations.

Test and Measurement

If test and measurement equipment is operated with unscreened cables, or used for measurements on open set-ups, the user has to assure that under operating conditions the radio interference limits are still met within the premises.

Sound Emission

Sound Pressure

Sound pressure Lp < 70 db(A) according to DIN EN ISO 7779

Schalldruckpegel

Schalldruckpegel Lp < 70 db(A) nach DIN EN ISO 7779

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In This Book

The manual describes the following:

- introduction,
- installing,
- configuring,
- using,
- data analysis,
- safety and related information.

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